PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/52, 15/53, 15/54
C12N 1/21, A01H 5/00
C12P 7/62

A1

(11) International Publication Number: WO 93/02194

(43) International Publication Date: 4 February 1993 (04.02.93)

(21) International Application Number: PCT/GB92/01291

(22) International Filing Date: 15 July 1992 (15.07.92)

(30) Priority data:
9115245.4 16 July 1991 (16.07.91) GB

(71) Applicant *(for all designated States except US)*: IMPERIAL CHEMICAL INDUSTRIES PLC [GB/GB]; Imperial Chemical House, Millbank, London SW1P 3JF (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : LIEBERGESELL, Mathias [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE). STEINBÜCHEL, Alexander [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE).

(74) Agent: HUSKISSON, Frank, Mackie; Imperial Chemical Industries plc, ICI Group Patent Department, P.O. Box 6, Bessemer Road, Welwyn Garden City, Herts AL7 1HD (GB).

(81) Designated States: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.

(54) Title: PRODUCTION OF POLYALKANOATE

(57) Abstract

Genes encoding polyhydroxyalkanoate synthase, β-ketothiolase and acetoacetyl CoA reductase are isolated from the publicly available bacterium *Chromatium vinosum*. Recombinant genomes of plants or other species of bacteria which contain these genes are capable of producing polyalkanoate polymers. The nucleotide sequences of the said three genes have been determined.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

PRODUCTION OF POLYALKANOATE

This invention relates to the production of polyhydroxyalkanoate by the culture of microorganisms which produce same.

Poly-3-hydroxybutyrate is a linear polyester of D(-)-3-hydroxybutyrate. It was first dicovered in Bacillus megaterium in 1925. Polyhydroxybutyrate accumulates in intracellular granules of a wide variety of bacteria. The granules appear to be membrane bound and can be stained with Sudan Black dye. The polymer is produced under conditions of nutrient limitation and acts as a reserve of carbon and energy. The molecular weight of the polyhydroxybutyrate varies from around 50,000 to greater than 1,000,000, depending on the microorganisms involved, the conditions of growth, and the method employed for extraction of the polyhydroxybutyrate. Polyhydroxybutyrate is an ideal carbon reserve as it exists in the cell in a highly reduced state, (it is virtually insoluble), and exerts negligible osmotic pressure.

Polyhydroxybutyrate and related poly-hydroxyalkanoates, such as poly-3-hydroxyvalerate and poly-3-hydroxyoctanoate, are biodegradable thermoplastics of considerable commercial importance.

The term "polyhydroxyalkanoate" as used hereinafter includes copolymers of polyhydroxybutyrate with other polyhydroxyalkanoates such as poly-3-hydroxyvalerate.

Polyhydroxyalkanoate is biodegradable and is broken down rapidly by soil microorganisms. It is thermoplastic (it melts at 180°C) and can readily be moulded into diverse forms using technology well-established for the other thermoplastics materials such as high-density polyethylene which melts at around the same temperature (190°C). The material is ideal for the production of biodegradable packaging which will degrade in landfill sites and sewage farms. The polymer is biocompatible, as well as biodegradable, and is well tolerated by the mammalian, including human, body, its degradation product, 3-hydroxybutyrate, is a normal mammalian metabolite. However, polyhydroxyalkanoate degrades only slowly in the body and its medical uses are limited to those applications where long term degradation is required.

Polyhydroxyalkanoate, produced by the microorganism Alcaligenes eutrophus, is manufactured, as a copolymer with of polyhydroxy-butyrate and polhydroxyvalerate, by Imperial Chemical Industries PLC and sold under the Trade Mark BIOPOL. It is normally supplied in the form of pellets for thermoprocessing. However, polyhydroxyalkanoate is more expensive to manufacture by existing methods than, say, polyethylene. It is, therefore, desirable that new, more economic production of polyhydroxy-alkanoate be provided.

An object of the present invention is to provide materials and a method for the efficient production of polyhydroxyalkanoate.

According to the present invention there is provided gene fragments isolated from the bacterium Chromatium vinosum and encoding PHA polymerase, acetoacetyl CoA reductase and β-ketothiolase.

Preferably the C.vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.

The invention also provides a 16.5kb EcoR1 fragment of C.vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoR1 fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain all three of said genes responsible for expression of PHAs.

The invention further provides a fragment of the said PP10 fragment, designated SE45, encoding the PHA-synthase and β-ketothiolase genes and a region, designated SB24, encoding the acetoacetyl CoA reductase gene.

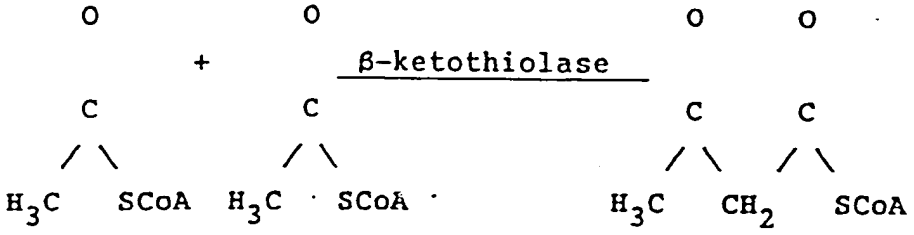
The invention also provides a recombinant genome of a microorganism, preferably a bacterium or a plant, which contains one or more of said fragments designated PP10, SE45 and region SB24.

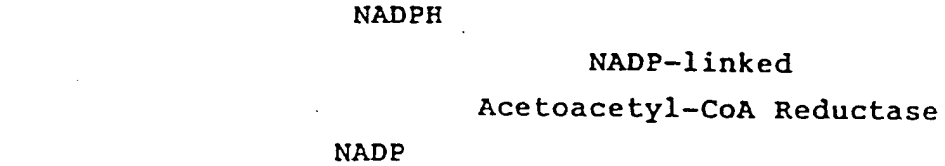
Finally, the invention provides a method for the manufacture of PHAs, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.

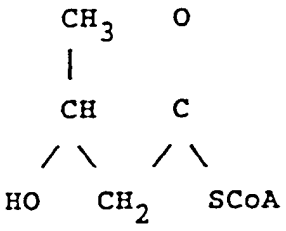
The biosynthesis of polyhydroxyalkanoate from the substrate, acetyl-CoA involves three enzyme-catalyse steps.

The three enzymes involved are β-ketothiolase, acetoactyl-CoA-reductase and polyhydroxy-

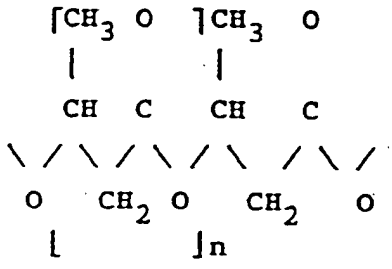
butyrate-synthase, the genes for which have been cloned from Chromatium vinosum. The three genes are known to facilitate production of polyhydroxyalkanoates, the reactions involved being represented as follows:.







Polyhydroxybutyrate-
synthase



The invention will now be described with reference to the accompnaying drawings, of which;

Figure 1 is the physical map of the 16.5 kb EcoR1 fragment of Chromatium vinosum DNA, designated PP10. The positions of the restriction sites and positions and names of the sub-fragments are shown. PHA-synthase and β-ketothiolase genes are located in fragment SE45 and acetoacetyl CoA reductase in region SB24;

Figure 2 is the map of PP10 showing the positions of the β-ketothiolase and acetoacetyl CoA reductase genes and of the PHA-synthase gene open reading frames ORF2 and ORF3.

Figure 3 is the complete nucleotide sequence of fragment SE45. The transcriptional start sites and terminators for the β-ketothiolase gene and for ORF3 and ORF 3 are shown. The positions of the "-10" and "-35" sequences are also shown, as are the positions of the putative ribosome binding sites ("s/d"). Translational start and stop (*) codon are also marked and the amino acid sequences of the β-ketothiolase, ORF2 and ORF3 are given.

Figure 4 shows the alignment of the amino acid sequences of Chromatium vinosum ORF3 with PHA polymerase of Alcaligenes eutrophus and PHA polymerases 1 and 2 of Pseudomonas oleovorans.

Figure 5 shows the complete nucleotide sequence of the DNA encoding PHA synthesis genes from Chromatium vinosum . The positions of PHA polymerase (phbC), acetoacetyl CoA reductase (phbB) and ketothiolase (phbA) genes are shown, and also the positions of ORF2, ORF4, ORF5 and ORF7.

Figure 6 shows the alignment of the amino acid sequences of ketothiolases encoded by C.vinsoum

(C.v.), A.eutrophus (A.e.), Zoogloea ramigera (Z.r.), Escherichia coli (E.c.), Saccharomyces uvarum (S.u.) and Rattus norvegicus (R.n.)

Figure 7 shows the alignment of the amino acid sequences of acetoacetyl CoA reductases encoded by C.vinosum (C.v.), A.eutrophus (A.e.) and Z.ramigera (Z.r.)

Figure 8 is a Table (Table 1) showing the heterologous expression in Escherichia coli of DNA fragments from C.vinosum. Activities of PHA biosynthetic enzymes expressed by the different fragments are shown. The levels of PHA accumulated in E.coli transformed with the fragments are also given.

EXAMPLE

The organism C.vinosum was a gift from Dr J. Imhoff of the University of Bonn, Germany.

1. Isolation of DNA fragments from C.vinosum encoding PHA synthesis genes

A 5.2 kb SmaI/EcoRI fragment (SE52), which codes for all three PHA biosynthetic genes has previously been isolated from Alcaligenes eutrophus [Schubert et al., J. Bacteriol. 170 (1988)]. This fragment was used to detect PHA biosynthetic genes of C.vinosum. EcoRI restricted genomic DNA of C.vinosum was blotted on to a nylon membrane and hybridized with biotinylated SE52 DNA. One signal appeared, representing a DNA fragment of 16.5 kb.

A λL47 gene bank from C.vinosum genomic DNA was prepared and plates with approximately 800 plaques were blotted on to nylon membranes and hybridized with biotinylated SE52 DNA. One positive recombinant phage was isolated, which harboured a 16.5 kb EcoRI fragment, which was

designated PP10 (Figure 1). With PP10 and a 9.4 kb EcoRI/PstI subfragment (EP94) of PP10, the phenotype of the wild type could be restored in PHA-negative mutants of A.eutrophus.

Expression studies in E.coli (see below) showed that a 4.5 kb SmaI/EcoRI (SE45) subfragment of EP94 encodes for PHA synthase and β-ketothiolase. The nucleotide sequence of this fragment was determined by the dideoxy-chain termination method of Sanger et al. with alkaline denatured double stranded plasmid DNA. The T7-polymerase sequencing kit of Pharmacia, Uppsala, Sweden, was used with 7-deazaguanosine-5'-triphosphate instead of dGTP. Most of the sequence was determined with a set of unidirectional overlapping deletion clones generated by exonuclease III digestion. For sequencing regions which were not covered by the deletion plasmids synthetic oligonucleotides were used.

It was not possible to clone the 4.9 kb SmaI/PstI fragment PS49 in a multi-copy vector. Therefore, fragment EP94 (Figure 1) was treated with Exonuclease Bal31, ligated to Bluescript SK and transferred to E.coli Xl-1 Blue. A clone was isolated which harboured Bluescript SK with a 5.5 kb fragment (B55) and which expressed β-ketothiolase and NADH-dependent reductase activity. 3146 base pairs of B55 were part of the SE45 fragment. The other part (approximately 2350 base pairs, SB24) has been sequenced applying the primer hopping strategy. The sequence and the position of the reductase gene on SB24 are known. The results of these studies, including the organisation of the PHA biosynthetic genes in

C.vinosum and the sites of the ketothiolase, reductase and PHA synthase genes are shown in Figure 2. The determination of the full sequence of SB24 is in progress.

2. Sequence analysis of the C.vinosum PHB Synthetic Genes

The nucleotide sequence of SE45 is shown in Figure 3. The fragment size of SE45 is 4506 bp.

2.1 PHB synthase

The fragment sequence corresponding to the PHB synthase gene is designated as ORF3. The determination of synthase activity of deleted plasmids containing SE45 (See below) gave evidence that expression of ORF2 is also required for expression of synthase activity.

ORF2 and ORF3 are transcribed as an operon. The determination of the transcription start site of ORF2 was conducted by S1 nuclease mapping. This site occurs at bp 3059 from the 3' end of SE45. A putative "-10" site, given as 5'-ACAGAT-3' occurs at bp 3073-3078, and a "-35" site occurs at bp 3092-3099. A putative ribosome binding site occurs at bp 3040-3045. The translation start codon commences at bp 3030. The translation stop codon occurs at bp 1958.

The putative ribosome binding site of ORF3 occurs at bp 1907-1911. The translation start ATG for ORF3 occurs at bp 1899, and the translation stop codon at bp 833. Putative transcriptional terminator sites occur at hairpin structures at bp 773-786 and 796-823.

ORF2 encodes a polypeptide of 358 amino acids with a MW of 40525 da. ORF3 encodes a polypeptide of 356 amino acids with a MW of 39739 da. The gene

size of ORF3 is approximately 30% smaller as compared with the PHA polymerase genes of A.eutrophus and P. oleovorans. The alignments of the primary structures of C.vinosum PHA polymerase, A.eutrophus PHA polymerase and P.oleovorans PHA polymerases 1 and 2 are shown in Figure 4. Thus the ORF3 C.vinosum polymerase is shorter than the other polymerase enzymes, lacking the first 172 amino acids from the NH_2 terminus of the A.eutrophus PHA polymerase, and the first 148 amino acids of the Pseudomonas polymerases. The amino acid sequence of ORF3 exhibited an overall homology of 25% to the polymerase of A.eutrophus, with certain discrete regions of conserved sequence.

The amino acid sequence of ORF2 showed no significant homology to other enzymes in the NBRF gene bank.

2.2 β ketothiolase

The β ketothiolase and acetoacetyl CoA reductase genes are transcribed in opposite direction to ORF2 and ORF3 (Figure 2). A "-10" site in the identified ketothiolase promoter occurs at bp 3105-3111, and a "-35" site at bp 3082-3086. A putative ribosome binding site occurs at bp 3167-3171. The translation starts signal occurs at bp 3181. The translation stop codon occurs at bp 4361.

The aligments of the primary structures of β ketothiolases from Chromatium vinosum and other sources are shown in Figure 5. Considerable homology is apparent betwen the amino acid sequences of ketothiolases from C.vinosum and other bacterial and non-bacterial sources.

2.3 Acetoacetyl CoA reductase

The alignments of the primary structures of acetoacetyl CoA reductases from C.vinosum, A.eutrophus and Z.ramigera are shown in Figure 6. All three reductases are of similar chain length, while considerable homology is apparent between the sequences of reductases from these bacteria.

The Chromatium vinosum PHA synthetic genes therefore differ from the PHA synthetic genes of A.eutrophus and P.oleovorans in the following respects:

i) Whereas A.eutrophus PHB polymerase, acetoacetyl CoA reductase and β ketothiolase genes are all transcribed as an operon, in C.vinosum the ketothiolase and reductase genes are transcribed separately from the polymerase, and are transcribed in the opposite direction to the polymerase ORF3 and ORF2 genes.

ii) In contrast to A.eutrophus, where one gene product is required for polymerase activity, in C.vinosum two gene products, represented by ORF2 and ORF3 are required for expression of polymerase activity.

iii) The C.vinosum ORF3 polymerase is 172 amino acids shorter, at the amino terminus, than the A.eutrophus polymerase, and 148 amino acids shorter than the P.oleovorans polymerases 1 and 2. The C.vinosum ORF3 shows only 25% homology with the primary sequence of the A.eutrophus polymerase.

iv) The A.eutrophus acetoacetyl CoA reductase enzyme involved in PHB synthesis is NADPH specific, while the C.vinosum enzyme exhibits a marked preference for NADH.

Between the structural genes for ketothiolase

and acetoacetyl CoA reductase of Chromatium vinosum, two open reading frames (ORF4 and ORF5) appear, and downstream from the reductase gene an ORF7 has been identified (Figure 5). No additional ORFs were identified in the PHA coding region of A.eutrophus.

3. Expression of C.vinosum PHB synthetic genes in other bacteria.

With fragments PP10 and EP94 the ability to synthesise PHB could be restored to PHB negative mutants of A.eutrophus. Recombinant strains of the PHB negative mutant A.eutrophus PHB-4, transformed with these fragments, were able to synthesise polymers containing 3-hydroxybutyrate and 3-hydroxyisovalerate at significant proportions, when supplied with appropriate substrates.

Studies on expression of C.vinosum DNA fragments in E.coli are presented in Table 1. Thus E.coli transformed with plasmids containing fragments PP10 and EP94 expressed PHB polymerase, acetoacetyl CoA reductase and β ketothiolase activities. They also synthesised PHB up to between 10 and 12% dry weight. E.coli transformed with plasmids containing fragment SE45 expressed PHB polymerase and β ketothiolase, but not acetoacetyl CoA reductase, and were unable to synthesise PHB.

4. Polymer Biochemistry

The specific optical rotations of methyl 3-hydroxybutyric acid liberated by methanolysis of PHB from C.vinosum (accumulated from acetate), from A.eutrophus PHB-4 pHP1014::PP10 (accumulated from fructose) and E.coli S17-1 pSUP202::PP10 (accumulated from glucose) were all negative. The

determined values of the specific optical rotation were similar to those for PHB isolated from A.eutrophus (accumulated from fructose).

CLAIMS

1. Gene fragments isolated from the bacterium Chromatium vinosum and encoding polyhydroxyalkanoate (PHA) synthase, acetoacetyl CoA reductase and β-ketothiolase.

2. Gene fragments as claimed in claim 1 in which the Chromatium vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.

3. A 16.5kb EcoR1 fragment of Chromatium vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoR1 fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain the genes encoding PHA-synthase acetoacetyl CoA reductase and β-ketothiolase.

4. A fragment of the PP10 fragment claimed in claim 3, designated SE45, encoding the PHA-synthase and β-ketothiolase genes.

5. A fragment of the PP10 fragment claimed in claim 3, designated SB24, encoding the acetoacetyl CoA reductase gene.

6. A recombinant genome which contains one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.

7. A bacterium having incorporated in its genome one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.

8. A plant having stably incorporated in its genome by transformation one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.

9. A method for the manufacture of polyhydroxyalkanoates, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.

10. A gene, encoding β-ketothiolase, having the nucleotide sequence shown in Figure 3.

11. A gene encoding polyhydroxyalkanoate synthase (phbC), having the nucleotide sequence shown in Figure 5.

12. A gene encoding acetoacetyl CoA reductase (phbB) having the nucleotide sequence shown in Figure 5.

FIG. 1

Physical map of the 16.5-kb *Eco*RI fragment PP10 and location of B55

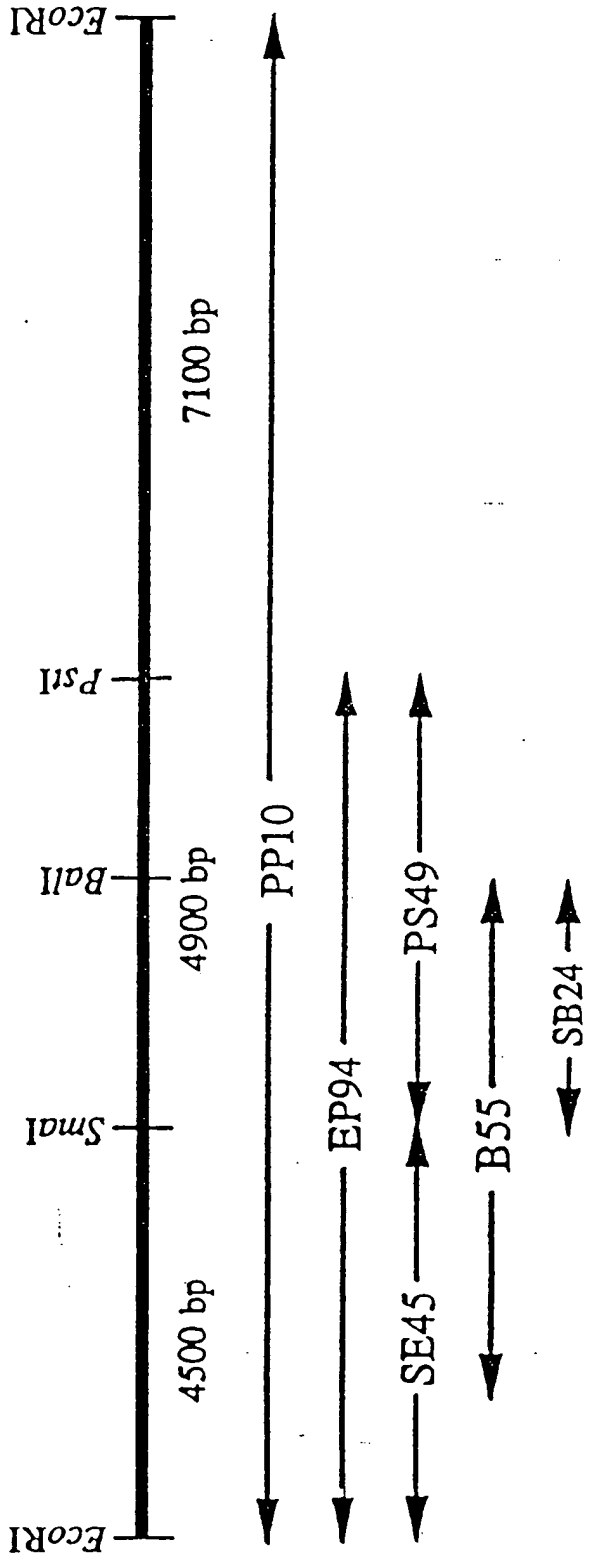


FIG. 2

Region of ß-ketothiolase and reductase locus and relevant open reading frames of PHB-synthase

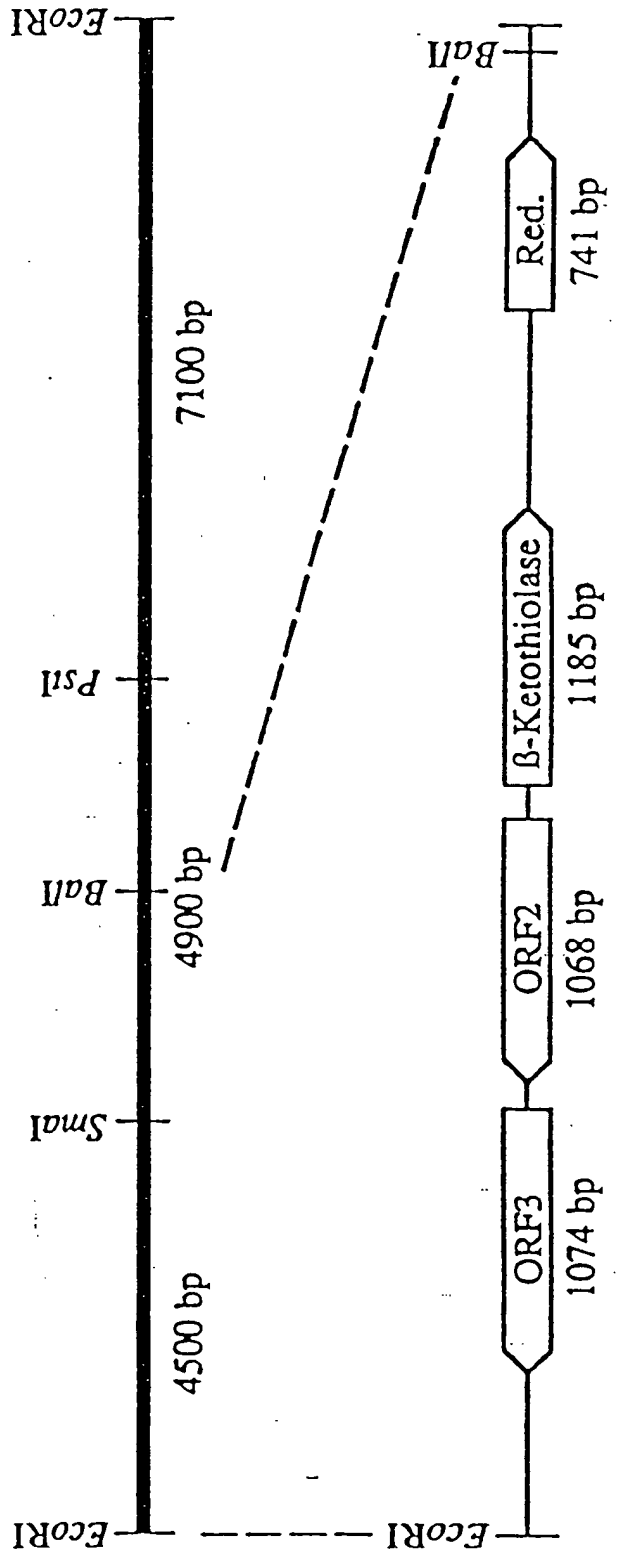


FIG. 3 (1/9)

: Nucleotide sequence of fragment SE45

*Eco*RI

```
   1  GCTTAAGTAGGTCCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACTTG

 101  CCGCGAAGTGTGCCCTGCCGACTAGGACTGAGGTAGGCTAGGCGTAGAG

 201  TCACGAGGTAACTAGGACCTCTTAGGTGTCGGCCCCCGCCCGCTGGCTC

 301  GGCAGGCCTAAGCGCTACTGCAGTCTCGCCCCGCAAAGAGCGTATAAGC

 401  GGGTGGGGCTACACAGCCCACAGCGCGCGGGGACAGCCCGACCGCGCGC

 501  ACGCCGCTCTGTGCAGGCGGGACCGAGACCCTGACCTTTGTCGTTCCTC

 601  ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCAGTCACCCTAGCC

 701  AATTGCACTTCCGGTCGAGGTTCAGGCGAGCTACTCGCTGCTGCGGGCC

 801  GCGGCCTTAACGAGGCCGCCGAGCCCTTCGCAATAGCGAGCAACTCGGT
                                    *  R  E  N  L  W

 901  CGGCTACACTGGCGGCCCCTTCCGGTCGAGCCACATCAGGCCCGACGAC
        G  H  I  G  G  P  F  A  L  E  T  Y  D  P  S  S

1001  TCGCGCTTCTACAACTCGTGGCCCGTCCACTACAGGAAGTCCAGGTGAA
        L  A  F  I  N  L  V  P  C  T  I  D  K  L  D  V
```

FIG. 3 (2/9)

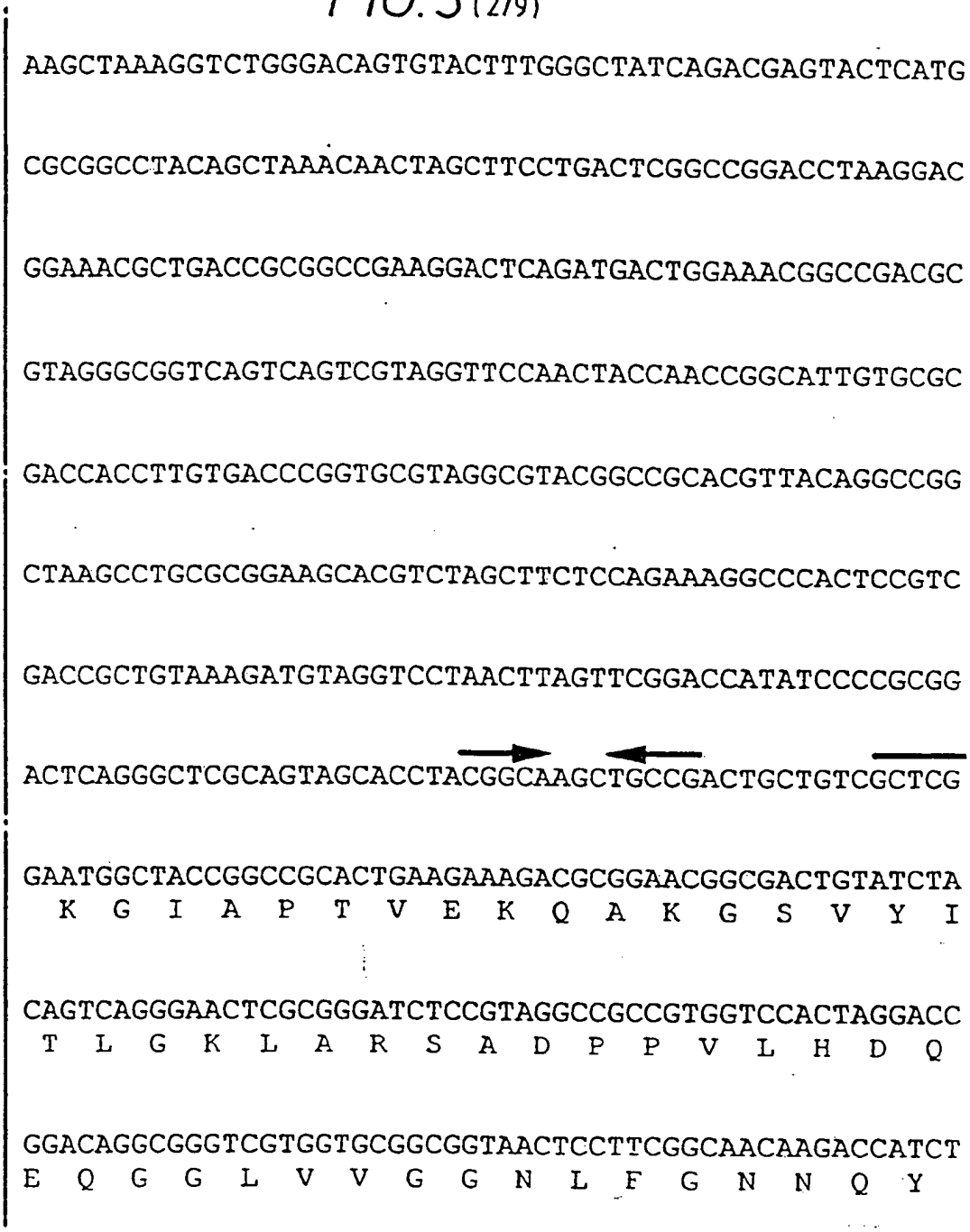

AAGCTAAAGGTCTGGGACAGTGTACTTTGGGCTATCAGACGAGTACTCATG
CGCGGCCTACAGCTAAACAACTAGCTTCCTGACTCGGCCGGACCTAAGGAC
GGAAACGCTGACCGCGGCCGAAGGACTCAGATGACTGGAAACGGCCGACGC
GTAGGGCGGTCAGTCAGTCGTAGGTTCCAACTACCAACCGGCATTGTGCGC
GACCACCTTGTGACCCGGTGCGTAGGCGTACGGCCGCACGTTACAGGCCGG
CTAAGCCTGCGCGGAAGCACGTCTAGCTTCTCCAGAAAGGCCCACTCCGTC
GACCGCTGTAAAGATGTAGGTCCTAACTTAGTTCGGACCATATCCCCGCGG
ACTCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTCGCTCG
GAATGGCTACCGGCCGCACTGAAGAAAGACGCGGAACGGCGACTGTATCTA
K G I A P T V E K Q A K G S V Y I
CAGTCAGGGAACTCGCGGGATCTCCGTAGGCCGCCGTGGTCCACTAGGACC
T L G K L A R S A D P P V L H D Q
GGACAGGCGGGTCGTGGTGCGGCGGTAACTCCTTCGGCAACAAGACCATCT
E Q G G L V V G G N L F G N N Q Y

FIG. 3 (3/9)

```
1101  TCAGGAACTACTTGACTGCCTTCCAGAGCGGCCGGACTAGGCCCGACA
        F  D  K  I  F  Q  R  F  T  E  G  A  Q  D  P  S

1201  CTCGTCTAGTTGGTACAACTGCATGAAGACTGGGCAGTCCGACTTTCC
        L  L  D  V  M  N  V  Y  K  Q  G  T  L  S  F  P

1301  CACAGCTGGCGGTCTAGCTACAGCTGCAAGACGTGGGTCCGGCTCTCG
        T  D  V  A  L  D  I  D  V  N  Q  V  W  A  S  L

1401  CGTGGAACAGGCCCACGTCGCGCGACATGTAGTCCGACTTCCGCGGCG
        R  V  K  D  P  H  L  A  S  Y  M  L  S  F  A  G

1501  TATCAGGTGCGTCGCCAGCTACATCGGCAACTACATCAGCAGCTCGCA
        Y  D  V  C  R  D  I  Y  G  N  I  Y  D  D  L  T

1601  ACCGGCCACCGGTCGTCAGGGAACTACCACCTCGCCAGAAGGACCTAC
        Q  G  T  A  L  L  G  K  I  T  S  R  D  E  Q  I

1701  CGACCCGAAGCCCGCGCGGGAGCCCAGCCAGTATCGCTATCTCCTGGT
        P  Q  A  E  P  A  G  E  P  R  D  Y  R  Y  L  V

1801  GAGCCGCAACTCGTCTAAGAGGTACGGGACTGGGTCGAACGCCGACAT
        E  A  N  L  L  N  E  M  G  Q  G  L  K  R  S  Y

                S/D
1901  TAGCGACAGGAGACTAAGACAGACCACTCGGACGACAAGCTAGTCGGC

2001  GGAACCGCCGGCCCCGCCCGCTGGACTCGCGTCACCGTCCGAACCACC
        A  K  A  A  P  A  P  S  R  L  A  T  A  P  K  T
```

FIG. 3 (4/9)

```
GCTTCTAGGTAAAGAGGTACGCGTCCTTCAAGAACTGGAACAGGCCCAGCAG
P  F  I  W  K  E  M  R  L  F  N  K  V  K  D  P  D  D

GAACTCGCTGTCCTTCCAGGTCAAGTCGTCGAGTGGCCCCTACAACGGGTAC
   K  L  S  L  F  T  W  N  L  L  E  G  P  I  N  G  M

TCCAACAGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
 L  N  D  P  T  K  F  D  V  P  T  V  M  T  V  L  N

GGACTGTCTATGGCTCGTCTAACTGGAACAGCTGCGGCACCCGAAGCGCGTC
G  Q  C  I  G  L  L  N  V  K  D  V  G  H  A  E  R  L

CTCGCGCGCCAGCGGGACTAGGCCTATCGGGGTCAGCTAGTCCATCTGCAGG
   L  A  R  D  A  Q  D  P  Y  G  W  D  I  L  Y  V  D

AGCCAGTACATCCCGGCCAACTGGTCCCGCATCTGCTAGTCCTCGCCGTGCC
 D  T  M  Y  P  R  N  V  L  A  Y  V  I  L  L  P  V

CGAACAGGAGCGACATCTGCCGGACGAACCCCGACTGCGGCCACAGCTACCG
L  K  D  E  S  Y  V  A  Q  K  P  S  V  G  T  D  I  A

                                              ORF3
CAGCTCGTAGAGGACCCACTCAAACAGGCCGGCCTACAGCTACCCCTTGTAC
   D  L  M  E  Q  T  L  K  D  P  R  I  D  I  P  F  M

GAACTGCGAGTTAGCCGGCCTAACCAGAACCACCACGCGGCCGCACGGCCTC
         *  D  A  P  N  T  K  T  T  R  R  R  A  P

GGCCGAGGAGTGGCCGGTCCCGACGCTGCGCTGCAAGGTCTAGCGCGTCCGA
A  P  E  E  G  A  L  A  A  V  R  R  E  L  D  R  L  S
```

FIG. 3 (5/9)

```
2101  CACTGCGTCCCGGAACAAGAGCGCTGCCCAGAGGACCTCGGCCAGGACC
        H  R  L  A  K  N  E  R  R  T  E  Q  L  R  D  Q

2201  AGCTGGTCCTAGCTGTACGCGAAGAACTCGCGGTAGACTCGCAACTGGT
        D  V  L  I  S  M  R  K  K  L  A  M  Q  A  N  V

2301  GCGGAAGCGTCGTCCGCTGGGTCAACAGCATCTCGCGCGCGCGGCTCAG
        A  G  E  C  C  A  V  W  N  D  Y  L  A  R  A  S  D

2401  CTGGCTGAAGTACGGCTCGAACCACATCTGCAACCACATAAGGACCTCG
        V  S  K  M  G  L  K  T  Y  V  N  T  Y  E  Q  L

2501  AGGAGCGCCCACATCGGCTCTGGCCCGCGGCTCTCCCGAGCTAGTTCTG
        E  E  R  T  Y  G  L  G  P  A  S  L  A  R  D  L

2601  CGCTGTAGCTCCTGTAGTACGCGACGGTCAACAGCTCGCCGTCGAGGGT
        P  S  M  S  S  M  M  R  Q  W  N  D  L  P  L  E  W

2701  CTTCGCGAAGACGTACAGAAGCTCGCAGAACCAGGTCTCGAGGTTCGGC
        F  R  K  Q  M  D  E  L  T  K  T  W  L  E  L  G

2801  TTCTTCAAGAACGGGACGAGGTAGTAGAAAAGGTACTTGCTCGCACTGT
        F  F  N  K  G  Q  E  M  M  K  E  M  F  S  R  S

2901  GGGTCCCGCACAACCGGCTTGACCTCCGCGACAGGTCTGGGTACCGGAA
        E  W  P  T  T  A  S  S  S  A  S  D  L  G  M  A  K

                          ORF2                    S/D
3001  TAGCAGTAACTTCTTTAATCACAACGAGTACCTGCATGGGAGGAGCACC
        D  D  N  F  F  N  T  N  S  M
```

FIG. 3 (6/9)

TCCCACGCGTCGAGCGACGCCCAGCCGTACAAGTTACGCGGGTCCAAGAGC
L T P L E S R T P M N L A G L N E

CGGCCGGCACCTACGCCCGCATAAGGCCACACCGCTGGAGCAGCCGTATCC
L R G H I R A Y E P T A V E D A Y

CTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGGTACACGAG
I T K G S D I V G Q I Y S G M H E

CGCCGGACTATGAGGTACCGCGACGCGTAGTCGAGGACTATGACCGAGACG
A A Q Y E M A S R M L E Q Y Q S Q

ACAGGAACTTGACGAGCACCCCGTACAACGCGTCGTACAGCGGGCCGTAGC
S D K F Q E H P M N R L M D G P M

CTTGCTGTAGTCCGCGACGTACCACAACGGCGGCAGCAGCTCGCTCGGCGA
F S M L R Q M T N G G D D L S G S

AACGGCCGCAACGGGAGCAGCCGCGCAAACCGCTTCCACAGCCGCTCTGCC
N G A N G E D A R K A F T D A L R

CTAGGCCCCGCCGCCCGCGGTACCGAAAGGTGGTGACTAGCTACCGGCGGA
L D P A A P A M A K W W Q D I A A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT
R S M D T W N D W Y K R Q L E L W

mRNA "-35" Ketothiolase
GCGCACCACAACCCGCTTGCCATAGTACACCTAAACACGTGACGTTGTTTC
"-10" ORF2 "-35"

FIG. 3 (7/9)

"-10"

```
3101  TACGCATCTTAGGTGTCCGGCGCTGGGGTATAGCCCCTGCGAGCAGGTA

          V  D  A  G  R  S  A  I  G  T  F  G  G  S  L  S
3201  GCAGCTGCGGCCGGCGTCACGGTAGCCTTGGAAGCCGCCGTCAGACAGC

          R  T  G  L  A  P  E  Q  I  D  E  V  I  L  G  Q  V
3301  GCATGGCCTGAGCGCGGCCTTGTCTAGCTGCTCCACTAAGAGCCGGTCC

             G  L  P  H  S  V  P  A  M  T  I  N  K  V  C  G
3401  GCCCCGATGGCGTAAGCCACGGCCGGTACTGGTAGTTGTTCCAGACGCC

          A  D  I  V  I  A  G  G  Q  E  S  M  S  Q  S  S
3501  ACGGCTGTAGCAGTAGCGGCCGCCAGTCCTCTCGTACTCGGTCAGGAGC

          K  D  T  M  I  V  D  G  L  W  D  A  F  N  N  Y  H
3601  TTCCTGTGGTACTAGCAGCTGCCGGAGACCCTACGGAAGTTGTTGATAG

             Q  Q  D  A  F  A  A  A  S  Q  Q  K  T  E  A  A
3701  TCGTCGTCCTGCGGAAGCGGCGGCGGAGCGTCGTCTTCTGGCTCCGGCG

          R  K  G  D  P  K  V  F  D  A  D  E  F  P  R  H
3801  CGCGTTCCCGCTAGGCTTCCACAAACTACGGCTGCTCAAGGGCGCAGTG

          G  S  V  T  A  G  N  A  S  G  I  N  D  G  A  A  M
3901  CCGTCGCAGTGCCGCCCATTGCGGAGGCCGTAGTTGCTGCCCCGCCGGT

             A  R  L  V  A  F  A  S  A  G  V  D  P  A  I  M
4001  ACCGCGCAGACCACCGGAAGCGGTCGCGGCCACAGCTAGGCCGCTAGTA
```

FIG. 3 (8/9)

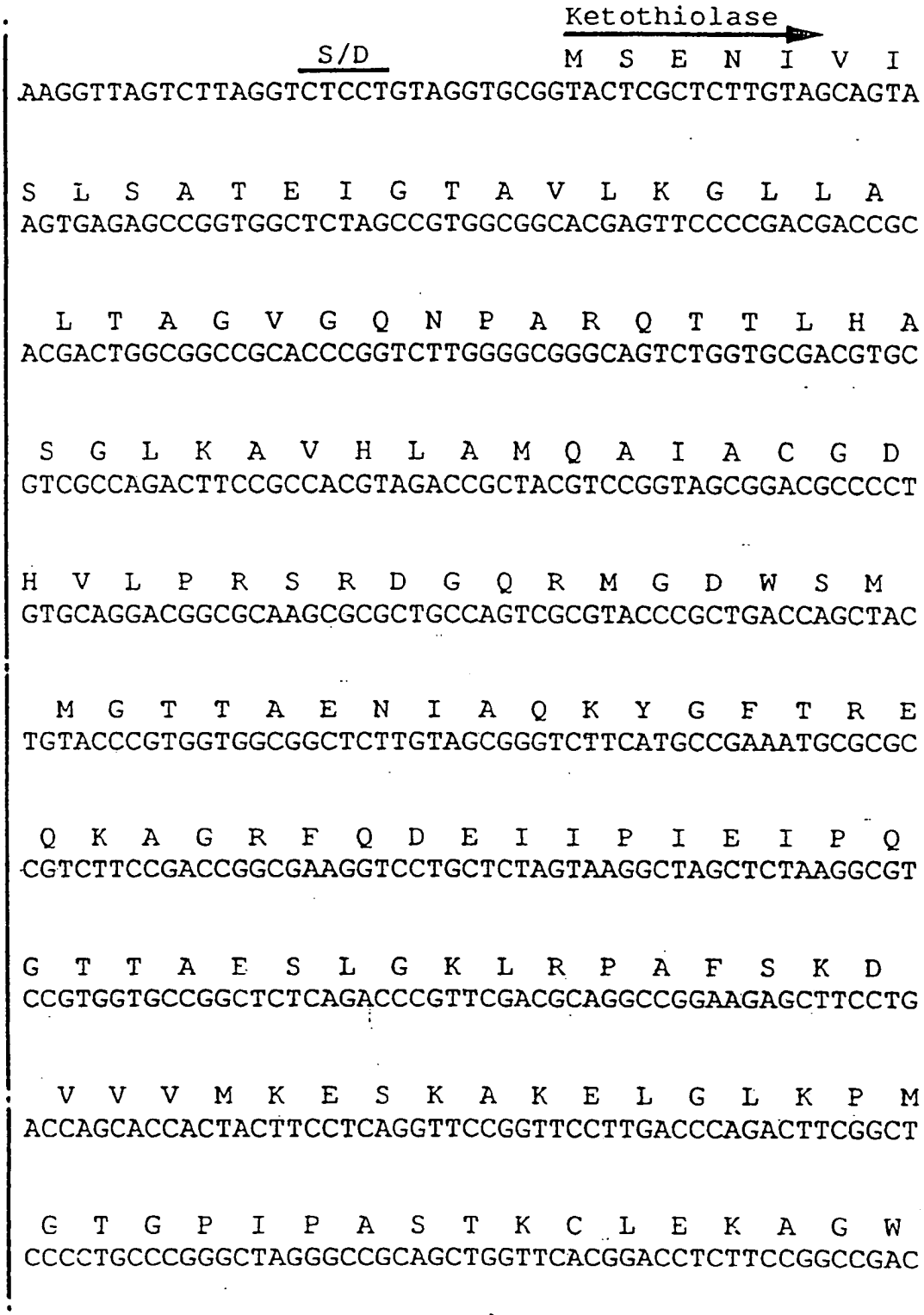

Ketothiolase
S/D
M S E N I V I
AAGGTTAGTCTTAGGTCTCCTGTAGGTGCGGTACTCGCTCTTGTAGCAGTA
S L S A T E I G T A V L K G L L A
AGTGAGAGCCGGTGGCTCTAGCCGTGGCGGCACGAGTTCCCCGACGACCGC
L T A G V G Q N P A R Q T T L H A
ACGACTGGCGGCCGCACCCGGTCTTGGGGCGGGCAGTCTGGTGCGACGTGC
S G L K A V H L A M Q A I A C G D
GTCGCCAGACTTCCGCCACGTAGACCGCTACGTCCGGTAGCGGACGCCCCT
H V L P R S R D G Q R M G D W S M
GTGCAGGACGGCGCAAGCGCGCTGCCAGTCGCGTACCCGCTGACCAGCTAC
M G T T A E N I A Q K Y G F T R E
TGTACCCGTGGTGGCGGCTCTTGTAGCGGGTCTTCATGCCGAAATGCGCGC
Q K A G R F Q D E I I P I E I P Q
CGTCTTCCGACCGGCGAAGGTCCTGCTCTAGTAAGGCTAGCTCTAAGGCGT
G T T A E S L G K L R P A F S K D
CCGTGGTGCCGGCTCTCAGACCCGTTCGACGCAGGCCGGAAGAGCTTCCTG
V V V M K E S K A K E L G L K P M
ACCAGCACCACTACTTCCTCAGGTTCCGGTTCCTTGACCCAGACTTCGGCT
G T G P I P A S T K C L E K A G W
CCCCTGCCCGGGCTAGGGCCGCAGCTGGTTCACGGACCTCTTCCGGCCGAC

FIG. 3 (9/9)

```
          T  P  A  D  L  D  L  I  E  A  N  E  A  F  A  A
4101  CTGGGGCCGCCTAGACCTAGACTAGCTCCGGTTGCTTCGGAAGCGGCGC

          V  N  G  G  A  I  A  I  G  H  P  I  G  A  S  G  A
4201  CAGTTGCCGCCGCGGTAGCGGTAGCCAGTAGGCTAGCCGCGGAGGCCAC

           G  L  A  T  L  C  I  G  G  G  Q  G  V  A  L  A
4301  TCCCAGACCGCTGCGACACGTAGCCGCCGCCGGTCCCGCACCGCGACCG

4401  TCGGAGGACTTAGCGAGGTCCGTGACTTGCGGGACGGCTAGGGCCTAGC

        SmaI
4501  GGGCCC
```

FIG. 3

```
Q  A  M  S  V  N  Q  D  M  G  W  D  L  S  K  V  N
GTCCGGTACAGCCAGTTGGTCCTGTACCCGACCCTAGACAGGTTCCAGTTG

  R  V  L  V  T  L  L  Y  E  M  Q  K  R  D  A  K  K
GCGCGCACGAGCACTGGGACGAGATACTCTACGTCTTCGCGCTGCGGTTCT

 V  E  R  M  *
CCAGCTCGCCTACACTCGGCAGCAGGCGGCCAGACTTAGCGGCCGCCTGGC

CACCCCGCAAACGCGCGAACCCCATCTGAACGGCTTGCTGGTCGGCTTGGC
```

FIG. 4 (1/3)

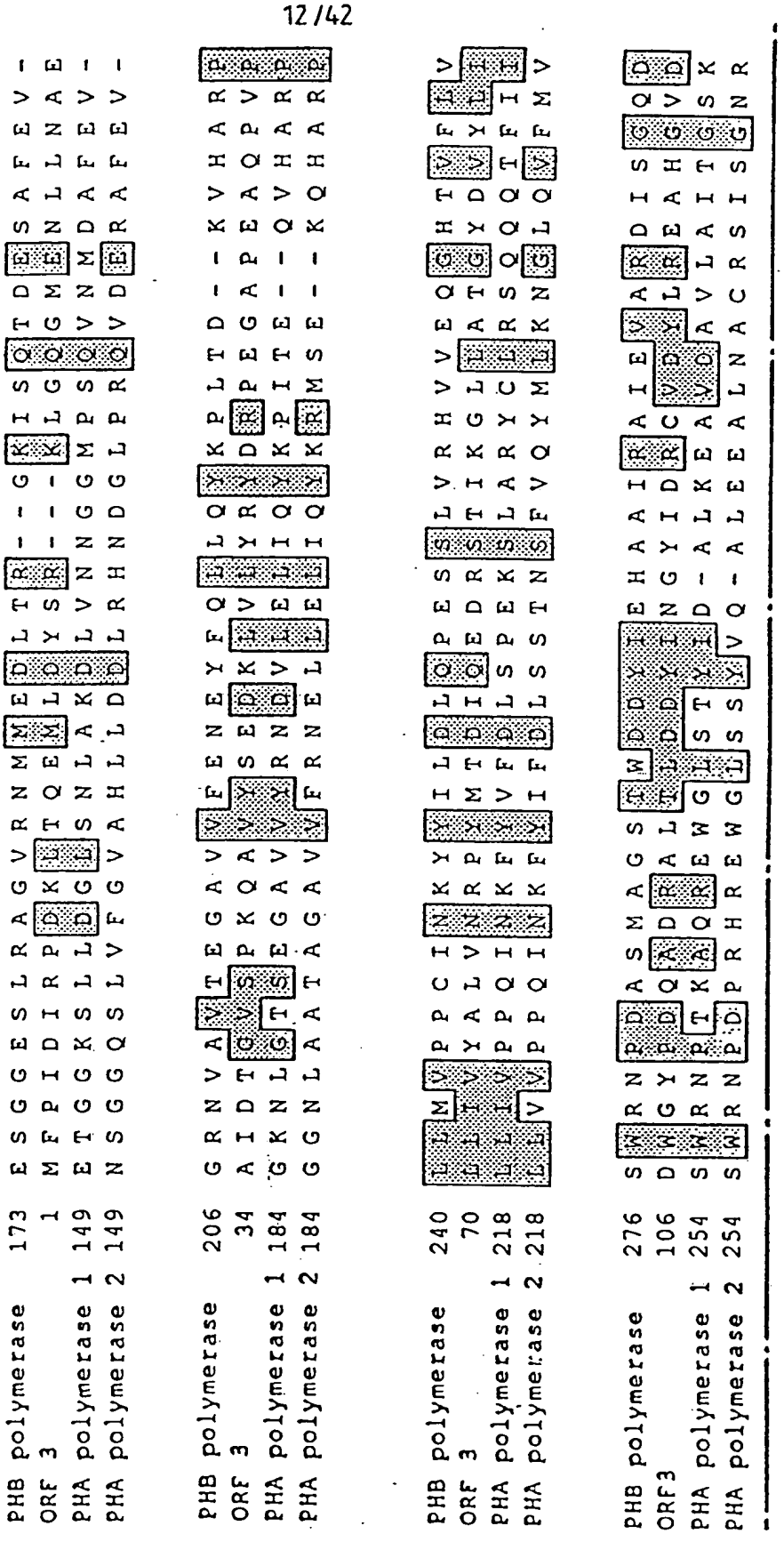

PHB polymerase 173 E S G G G E S L R A G V R N M M E D L T R - - G K I S Q T D E S A F E V -
ORF 3 1 M F P I D I R P D K L T Q E M L D Y S R - - - K L G Q G M E N L L N A E
PHA polymerase 1 149 E T G G K S L L D G L S N L A K D L V N N G G M P S Q Q V N M D A F E V -
PHA polymerase 2 149 N S G G Q S L V F G V A H L L D D L R H N D G L P R Q V D E R A F E V -
PHB polymerase 206 G R N V A V T E G A V V F E N E Y F Q E L Q Y K P L T D - - K V H A R P
ORF 3 34 A I D T G V S P K Q A V Y S E D K L V H Y R Y D R P E G A P E A Q P V P
PHA polymerase 1 184 G K N L G T S E G A V V Y R N D V L E L I Q Y K P I T E - - Q V H A R P
PHA polymerase 2 184 G G N L A A T A G A V V F R N E L L E L I Q Y K R M S E - - K Q H A R P
PHB polymerase 240 L L M V P P C I N K Y Y I L D L Q P E S S L V R H V V E Q G H T V F T V
ORF 3 70 L I T V Y A L V N R P X M T D I Q E D R S S T I K G L L A T G Y D V Y L L T
PHA polymerase 1 218 L L I V P P Q I D K F Y V F D L S P E K S L A R Y C L R S Q Q T F I
PHA polymerase 2 218 L L V P P Q I N K F Y I F D L S S T N S F V Q Y M L K N G L Q V F M V
PHB polymerase 276 S W R N R D A S M A G S T W D D Y I E H A A I R A I E V A R D I S G Q D
ORF3 106 D W G Y P D Q A D R A L T L D D Y I N G Y I D R C V D Y L R E A H G V D
PHA polymerase 1 254 S W R N R T K A Q R E W G L S T Y I D - A L K E A V D A V L A I T G S K
PHA polymerase 2 254 S W R N P D P R H R E W G L S S Y V Q - A L E E A L N A C R S I S G N R

FIG. 4(2/3)

```
PHB polymerase          312  K I N V L G F C V G G T I V S T A L A V L A A R G E - H P A A S V T L L
ORF3                    142  K V N L L G I C Q G G A F - - - S L M Y S A L H P D - K V R N L V T M V
PHA polymerase 1 288    D L N M L G A C S G G I T C T A L V G H Y A A L G E - N K V N A L I L L
PHA polymerase 2 288    D P N L M G A C A G G L T M A A L Q G H L Q A K H Q L R R V R S A T Y L

PHB polymerase          347  T T L L D F - A D T G I L D V F V D E G H V Q L R E A T L G G G A G A P
ORF3                    174  T P - V D F K T P D N L L S A W V Q N V D I D L A V D I M G N I P G E -
PHA polymerase 1 323    V S V L D T - T M D N Q V A L F V D E Q T L E A - - - - - A K R H S Y Q
PHA polymerase 2 324    V S L L D S - K F E S P A S L F A D E Q T I E A - - - - - A K R R S Y Q

PHB polymerase          382  C A L L R G L E L A N T F S F L R P N D L V W N Y V V D N Y L K G N T P
ORF3                    208  - L L N W T F L S L K P F S L T G Q K Y V N M V D L L D D P D K V K N F
PHA polymerase 1 353    A G V L E G S E M A K V F A W M R P N D L I W N Y W V N N Y L L G N E P
PHA polymerase 2 354    R G V L D G A E V A R I F A W M R P N D L I W N Y W V N N Y L L G K T P

PHB polymerase          418  V P F D L L F W N G D A T N L P G P W Y C W Y L R H T Y L Q N E L K V P
ORF3                    243  L R M E K - - W I F D S P D Q A G E T F R Q F I K D F Y Q N N G F - L N
PHA polymerase 1 389    P V F D I L F W N N D T T R L P A A F H G - D L I E M F K S N P L T R P
PHA polymerase 2 390    P A F D I L Y W N A D S T R L P A A L H G - D L L D F F K L N P L T H P
```

FIG. 4 (3/3)

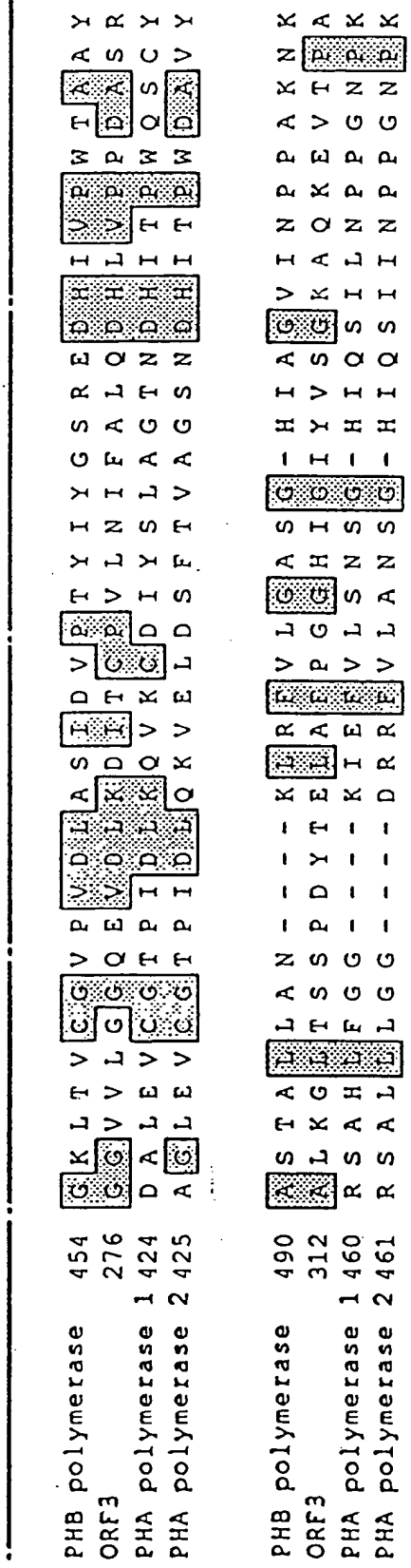

PHB polymerase 454 C K L T V C G V P V D L A S I D V P T Y I Y G S R E D H I V P W T A A Y
ORF3 276 G G V V L G Q E V D L K D I T G P V L N I F A L Q D H L V P P D A S R
PHA polymerase 1 424 D A L E V C G T P I D L K Q V K C D I Y S L A G T N D H I T P W Q S C Y
PHA polymerase 2 425 A G L E V C G T P I D L Q K V E L D S F T V A G S N D H I T P W D A V Y
PHB polymerase 490 A S T A H L A N - - - - K L R F V L G A S G - H I A G V I N P P A K N K
ORF3 312 R L K G J J T S S P D Y T E I A F P G G H I G I Y V S G K A Q K E V T P A
PHA polymerase 1 460 R S A H J F G G - - - - K I E F V L S N S G - H I Q S I L N P P G N P K
PHA polymerase 2 461 R S A L J L G G - - - - D R R F V L A N S G - H I Q S I I N P P G N P K
PHB polymertase 521 R S H W T N D A
ORF3 348 I G K W L N E R
PHA polymerase 1 491 A R F M T G A D
PHA polymerase 1 492 A Y Y L A N P K

FIG.5 (1/20)

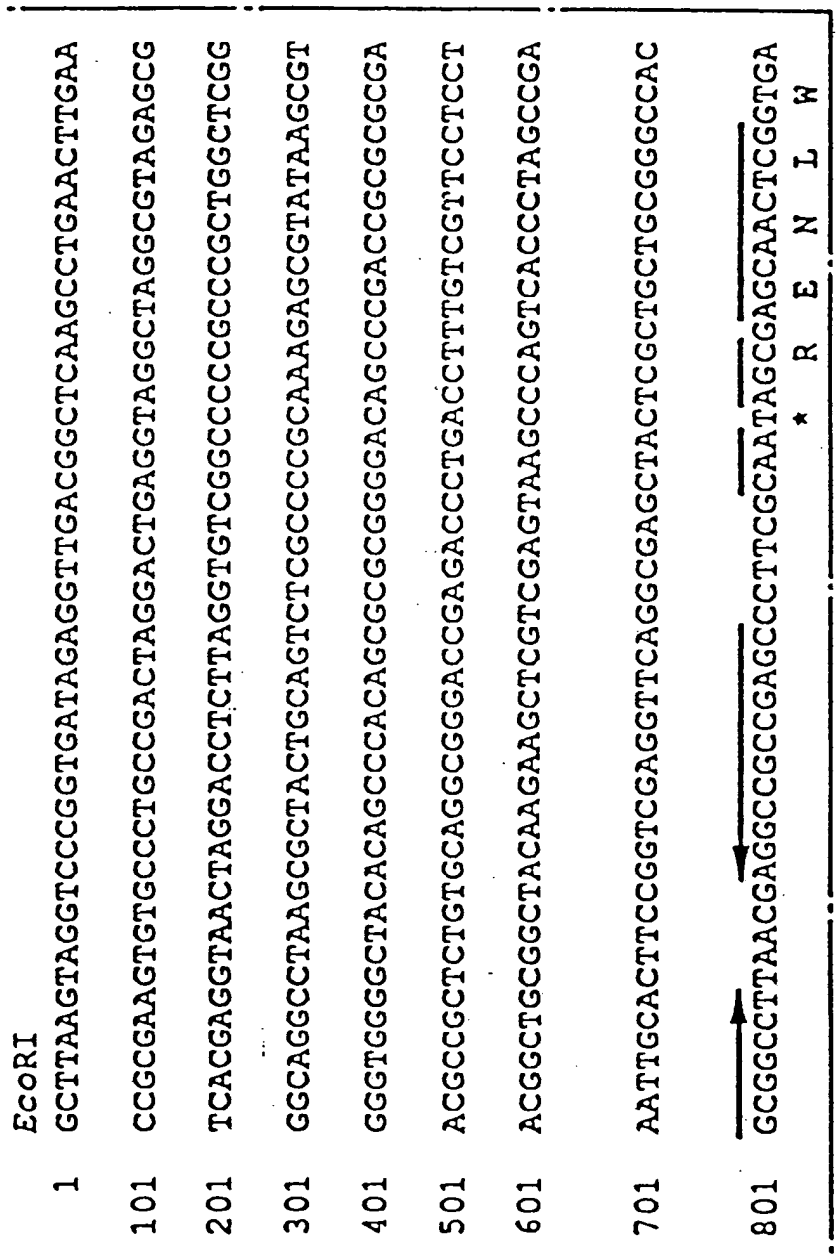

EcoRI
1 GCTTAAGTAGGTCCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACTTGAA
101 CCGCGAAGTGTGCCCTGCCGACTAGGACTGAGGTAGGCTAGGCGTAGAGCG
201 TCACGAGGTAACTAGGACCTCTTAGGTGTCGGCCCCCGCCCGCTGGCTCGG
301 GGCAGGCCTAAGCGCTACTGCAGTCTCGCCCCCGCAAGAGCGTATAAGCGT
401 GGGTGGGGCTACACAGCCCACAGCGCGCGGGGACAGCCCGACCGCGCGCGA
501 ACGCCGCTCTGTGCAGGCGGGACCGAGACCCTGACCTTTGTCGTTCCTCCT
601 ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCAGTCACCCTAGCCGA
701 AATTGCACTTCCGGTCGAGGTTCAGGCGAGCTACTCGCTGCTGCGGGCCAC
801 GCGGCCTTAACGAGGCCGCCGAGCCCTTCGCAATAGCGAGCAACTCGGTGA
* R E N L W

FIG.5 (2/20)

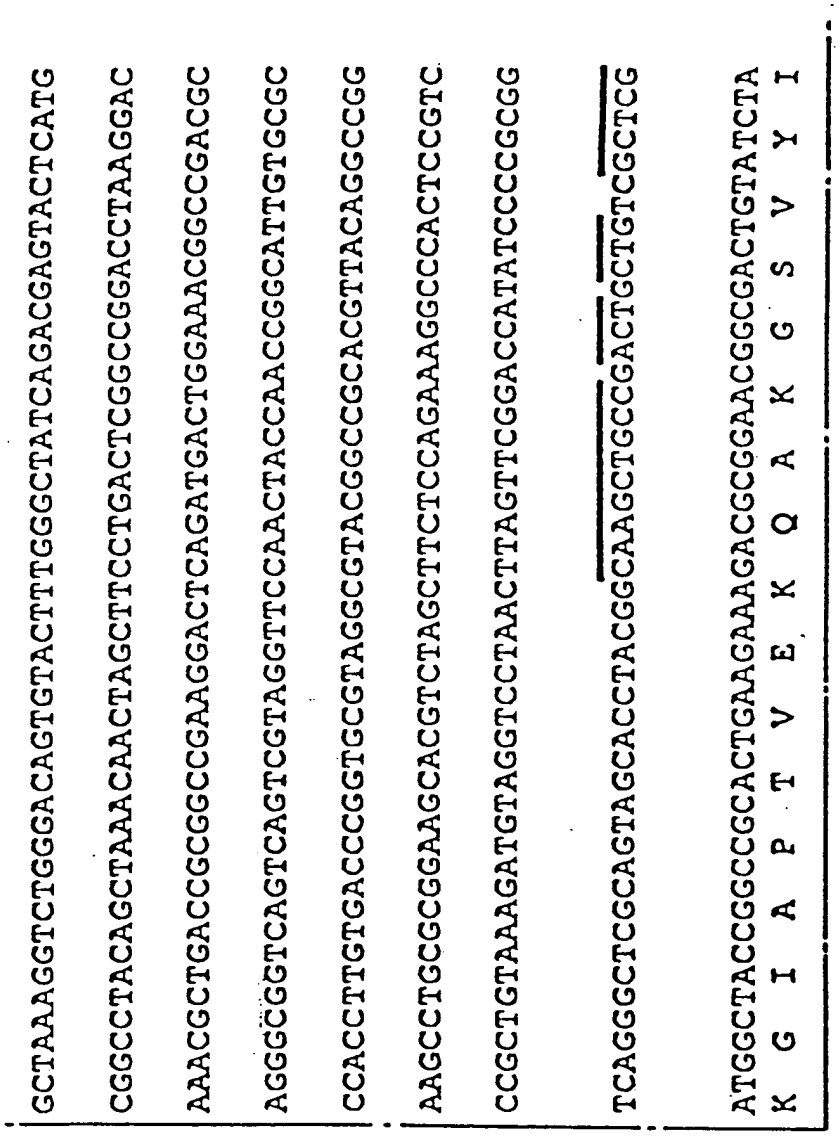

GCTAAAGGTCTGGGACAGTGTACTTTGGGCTATCAGACGAGTACTCATG
CGGCCTACAGCTAAACAACTAGCTTCCTGACTCGGCCGGACCTAAGGAC
AAACGCCTGACCGCGGCCGAAGGACTCAGATGACTGGAAACGGCCGACGC
AGGGCGGTCAGTCGTAGGTTCCAACTACCAACCGGCATTGTGCGC
CCACCTTGTGACCCGGTGCGTAGGCGTACGGCCGCACGTTACAGGCCGG
AAGCCTGCGCGGAAGCACGTCTAGCTTCTCCAGAAGGCCCACTCCGTC
CCGCTGTAAAGATGTAGGTCCTAACTTAGTTCGGACCATATCCCCGCGG
TCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTCGCTCG
ATGGCTACCGGCCGCACTGAAGAAAGACGCGGAACGGCGACTGTATCTA
K G I A P T V E K K Q A K G S V Y I

FIG. 5 (3/20)

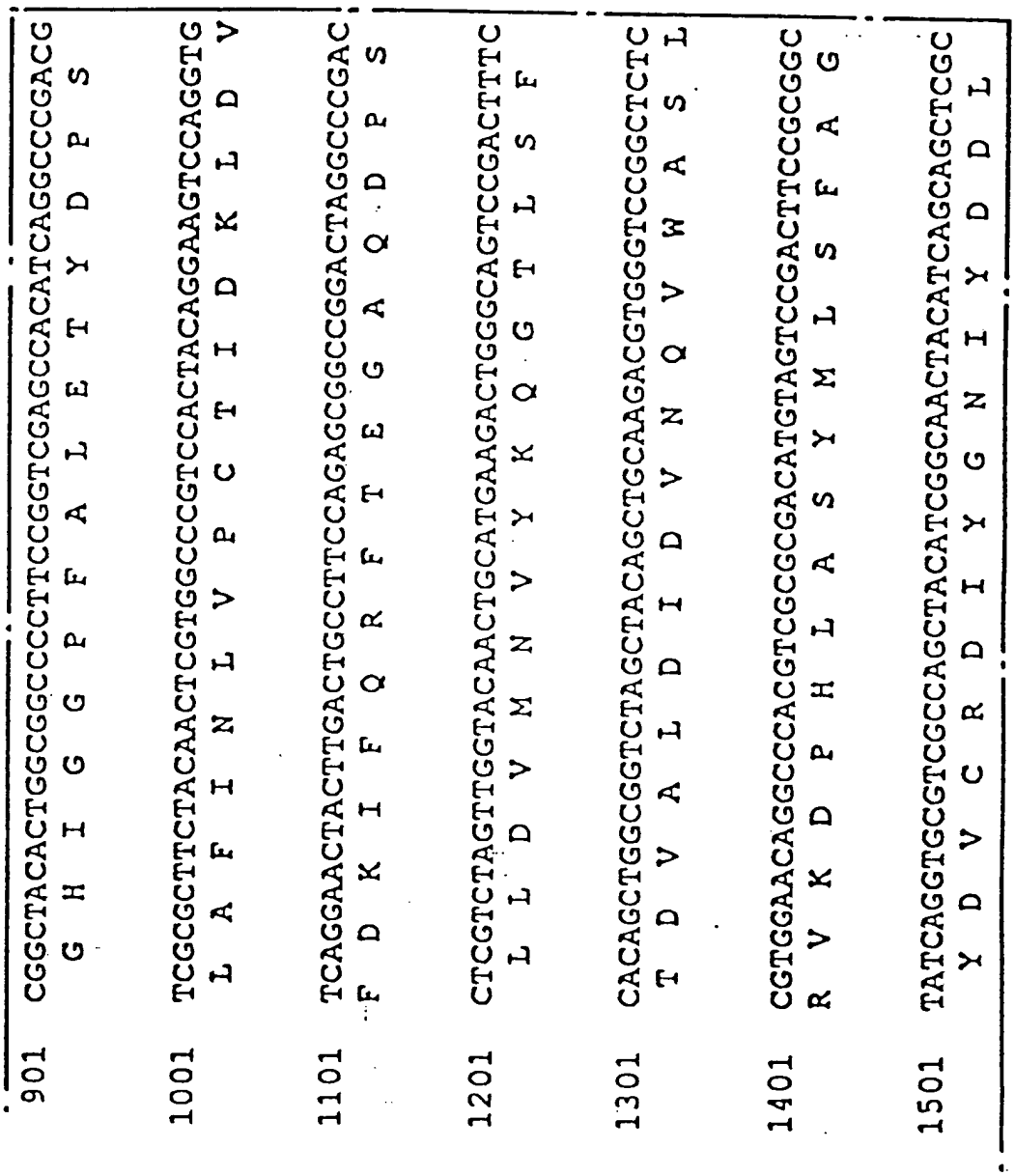

901 CGGCTACACTGGCGGCCCCTTCCGGTCGAGCCACATCAGGCCCGACG
G H I G G P F A L E T Y D P S
1001 TCGCGCTTCTACAACTCGTGGCCCGTCCACTACAGGAAGTCCAGGTG
L A F I N L V P C T I D K L D V
1101 TCAGGAACTACTTGACTGCCTTCCAGAGCGGCCGGACTAGGCCCGAC
F D K I F Q R F T E G A Q D P S
1201 CTCGTCTAGTTGGTACAACTGCATGAAGACTGGGCAGTCCGACTTTC
L L D V M N V Y K Q G T L S F
1301 CACAGCTGGCGGTCTAGCTACAGCTGCAAGACGTGGGTCCGGCTCTC
T D V A L D I D V N Q V W A S L
1401 CGTGGAACAGGCCCACGTCGCGGACATGTAGTCCGACTTCCGCGGC
R V K D P H L A S Y M L S F A G
1501 TATCAGGTGCGTCGCCAGCTACATCGGCAACTACATCAGCAGCTCGC
Y D V C R D I Y G N I Y D D L

FIG. 5 (4/20)

```
ACCAGTCAGGGAACTCGCGGGATCTCCGTAGGCCGCCGTGGTCCACTAGGACC
S  T  L  G  K  L  A  R  S  A  D  P  P  V  L  H  D  Q

AAGGACAGGCGGGTCGTGGTGCGGCGGTAACTCCTTCGGCAACAAGACCATCT
   E  Q  G  G  L  V  V  G  G  N  L  F  G  N  N  Q  Y

AGCTTCTAGGTAAAGAGGTACGCGTCCTTCAAGAACTGGAACAGGCCCAGCAG
P  F  I  W  K  E  M  R  L  F  N  K  V  K  D  P  D  D

CGAACTCGCTGTCCTTCCAGGTCAAGTCGTCGAGTGGCCCCTACAACGGGTAC
P  K  L  S  L  F  T  W  N  L  L  E  G  P  I  N  G  M

GTCCAACAGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
   L  N  D  P  T  K  F  D  V  P  T  V  M  T  V  L  N

GGGACTGTCTATGGCTCGTCTAACTGGAACAGCTGCGGCACCCGAAGCGCGTC
G  Q  C  I  G  L  L  N  V  K  D  V  G  H  A  E  R  L

ACTCGCGCGCCAGCGGGACTAGGCCTATCGGGGTCAGCTAGTCCATCTGCAGG
T  L  A  R  D  A  Q  D  P  Y  G  W  D  I  L  Y  V  D
```

FIG. 5 (5/20)

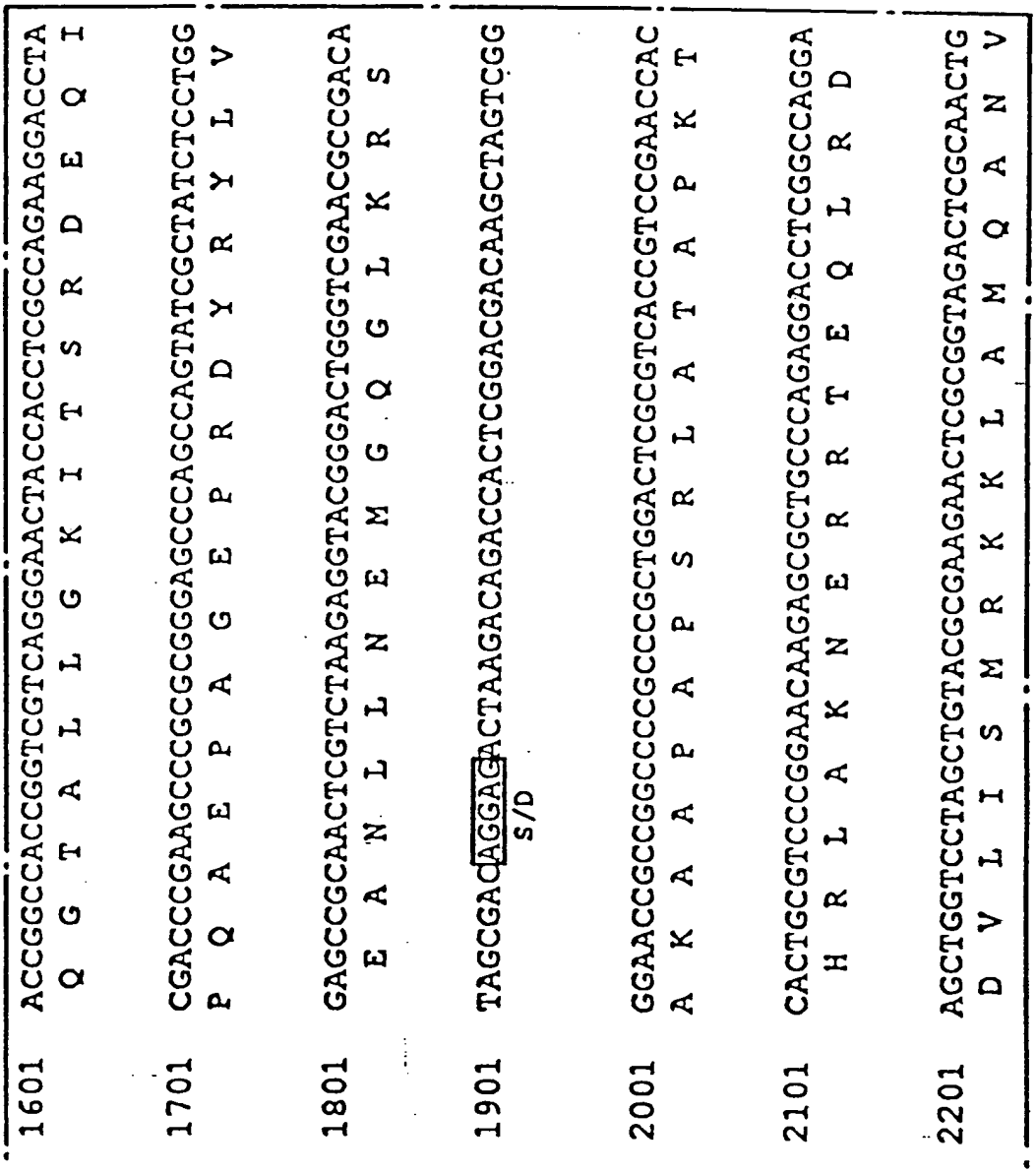

1601 ACCGGCCACCGGTCGTCAGGGAACTACCACCTCGCCAGAAGGACCTA
Q G T A L L G K I T S R D E Q I
1701 CGACCCGAAGCCCGCGCGGGAGCCCAGCCAGTATCGCTATCTCCTGG
P Q A E P A G E P R D Y R Y L V
1801 GAGCCGCAACTCGTCTAAGAGGTACGGGACTGGGTCGAACGCCGACA
E A N L L N E M G Q G L K R S
1901 TAGCGACAGGAGACTAAGACAGACCACTCGGACGACAAGCTAGTCGG
S/D
2001 GGAACCGCCGGCCCCGCCCGCTGGACTCGCGTCACCGTCCGAACCAC
A K A A P A P S R L A T A P K T
2101 CACTGCGTCCCGGAACAAGAGCGCTGCCCAGAGGACCTCGGCCAGGA
H R L A K N E R R T E Q L R D
2201 AGCTGGTCCTAGCTGTACGCGAAGAACTCGCGGTAGACTCGCAACTG
D V L I S M R K K L A M Q A N V

FIG. 5 (6/20)

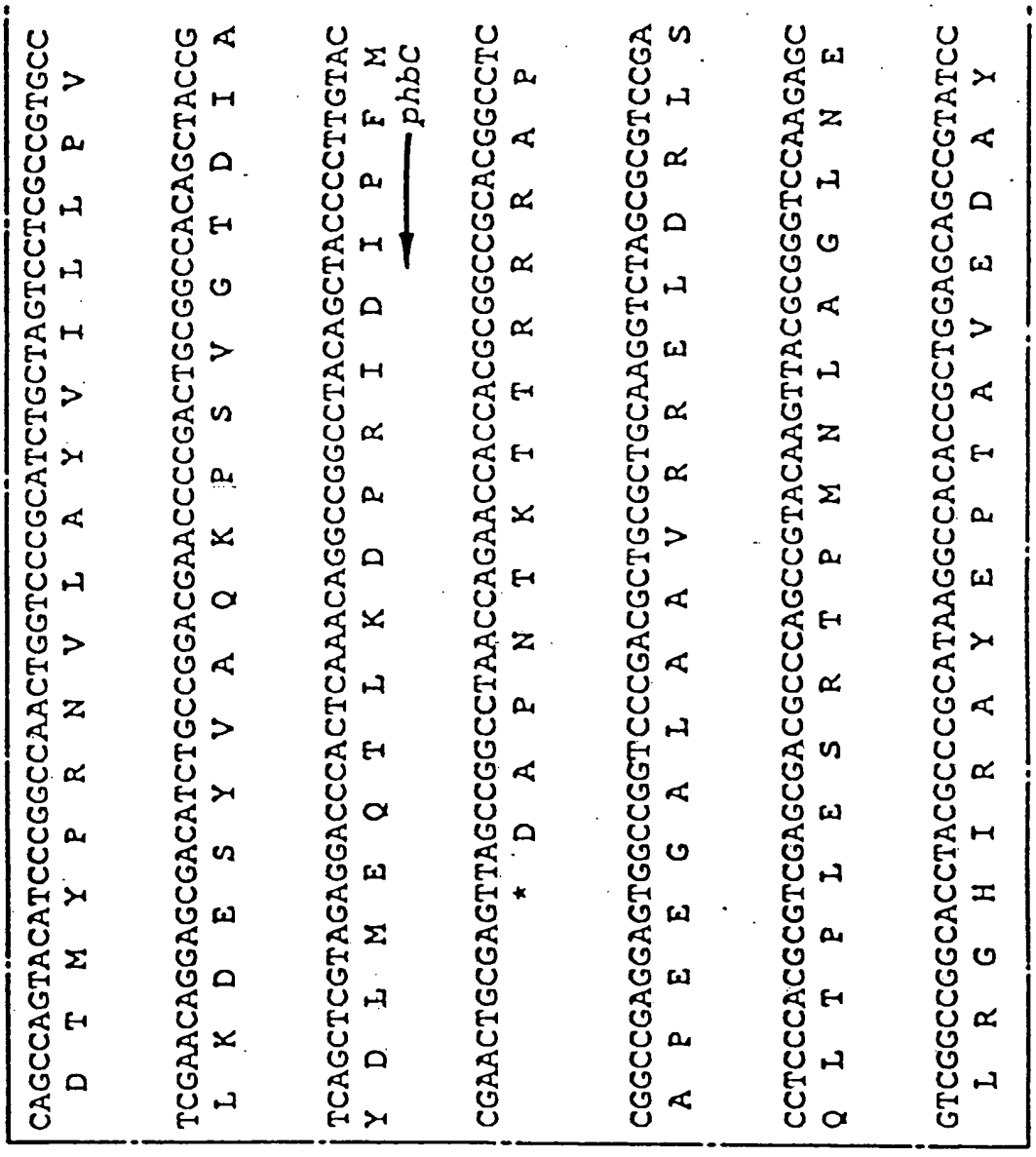

CAGCCAGTACATCCCGGCCAACTGGTCCCGCATCTGCTAGTCCTCGCCGTGCC
D T M Y P R N V L A Y V I L L P V
TCGAACAGGAGCGACATCTGCCGGACGAACCCCGACTGCGGCCACAGCTACCG
L K D E S Y V A Q K P S V G T D I A
TCAGCTCGTAGAGGACCCACTCAAACAGGCCGGCCTACAGCTACCCCTTGTAC
Y D L M E Q T L K D P R I D I P F M
phbC
CGAACTGCGAGTTAGCCGGCCTAACCAGAACCACCACGCGGCCGCACGGCCTC
* D A P N T K T T R R R A P
CGGCCGAGGAGTGGCCGGTCCCGACGCTGCGCTGCAAGGTCTAGCGCGGTCCGA
A P E E G A L A A V R R E L D R L S
CCTCCCACGCGTCGAGCGACGCCCAGCCGTACAAGTTACGCGGGTCCAAGAGC
Q L T P L E S R T P M N L A G L N E
GTCGGCCGGCACCTACGCCCGGCATAAGGCCACACCGCTGGAGCAGCCGTATCC
L R G H I R A Y E P T A V E D A Y

FIG. 5 (7/20)

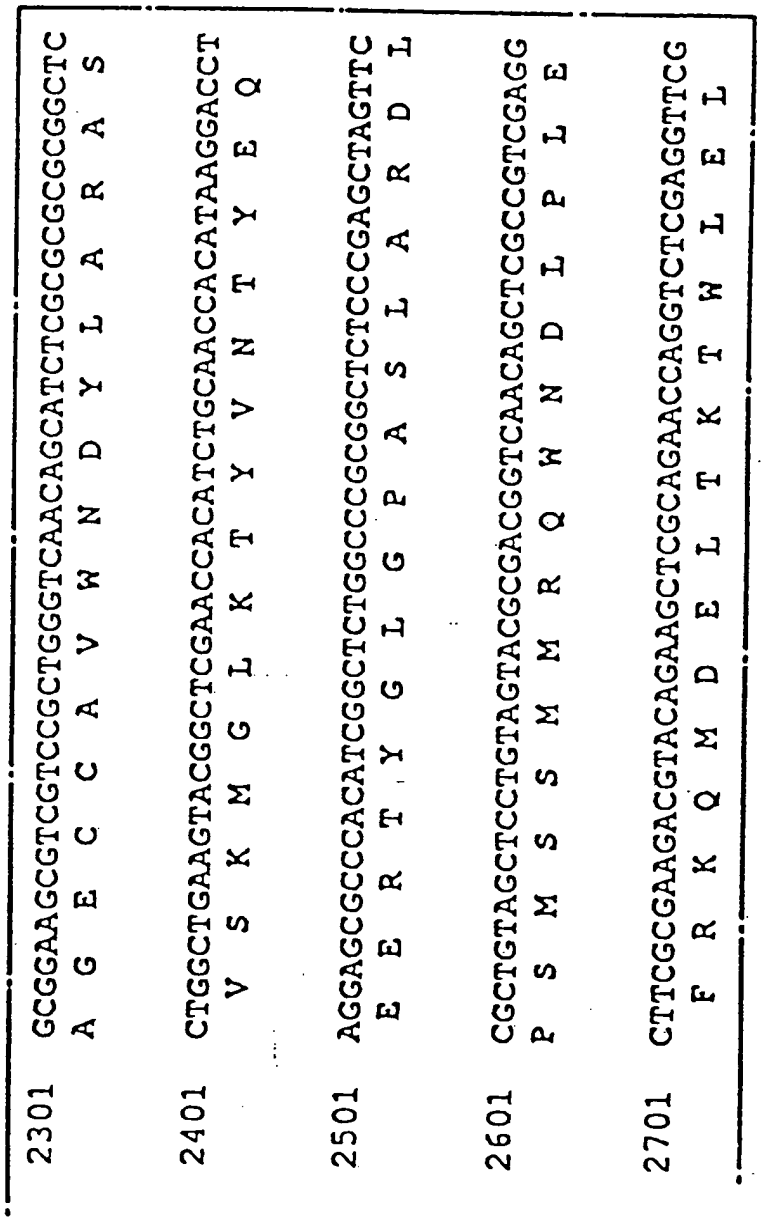

2301 GCGGAAGCGTCGTCCGCTGGGTCAACAGCATCTCGCGCGCGGCGGCTC
A G E C C A V W N D Y L A R A S
2401 CTGGCTGAAGTACGGCTCGAACCACATCTGCAACCACATAAGGACCT
V S K M G L K T Y V N T Y E Q
2501 AGGAGCGCCCACATCGGCTCTGGCCCGCGGCTCTCCCGAGCTAGTTC
E E R T Y G L G P A S L A R D L
2601 CGCTGTAGCTCCTGTAGTACGCGACGGTCAACAGCTCGCCGTCGAGG
P S M S S M M R Q W N D L P L E
2701 CTTCGCGAAGACGTACAGAAGCTCGCAGAACCAGGTCTCGAGGTTCG
F R K Q M D E L T K T W L E L

FIG.5 (8/20)

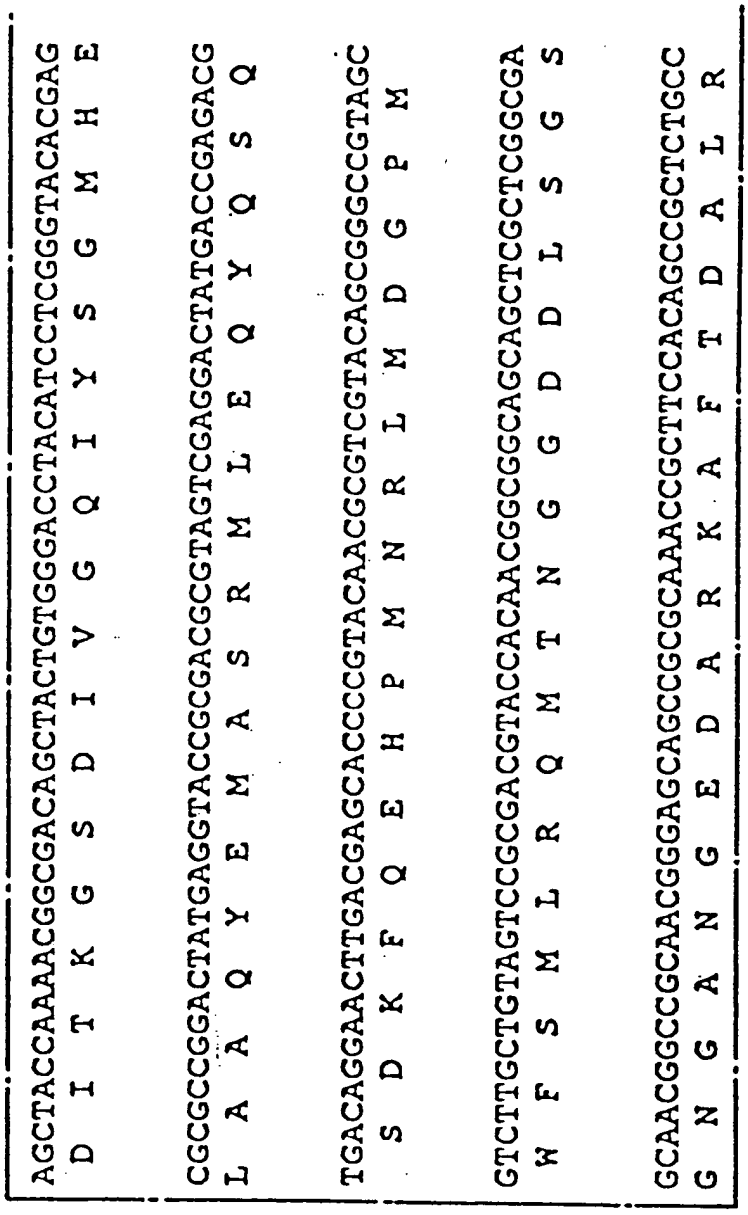

AGCTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGGTACACGAG
D I T K G S D I V G Q I Y S G M H E
CGCGCCGGACTATGAGGTACCGCGACGCGTAGTCGAGGACTATGACCGAGACG
L A A Q Y E M A S R M L E Q Y Q S Q
TGACAGGAACTTGACGAGCACCCCGTACAACGCGTCGTACAGCGGGCCGTAGC
S D K F Q E H P M N R L M D G P M
GTCTTGCTGTAGTCCGCGACGTACCACACAACGGCGGCAGCTCGCTCGGCGA
W F S M L R Q M T N G G D D L S G S
GCAACGGCCGCAACGGGAGGCAGCCGCGCGGCAAACCGCTTCCACAGCCGCTCTGCC
G N G A N G E D A R K A F T D A L R

FIG. 5(9/20)

```
2801  TTCTTCAAGAACGGGACGAGGTAGTAGAAAAGGTACTTGCTCGCACTGT
        F  F  N  K  G  Q  E  M  M  K  E  M  F  S  R  S

2901  GGGTCCCGCACAACCGGCTTGACCTCCGCGACAGGTCTGGGTACCGGAA
        E  W  P  T  T  A  S  S  S  A  S  D  L  G  M  A  K

3001  ATCGTCATTGAAGAAATTAGTGTTGCTCATGGACGTACCCTCCTCGTGG
      TAGCAGTAACTTCTTTAATCACAACGAGTACCTGCATGGGAGGAGCACC
        D  D  N  F  F  N  T  N  S  M          S/D
                                  ORF2
                    "-10"  mRNA
3101  ATGCGTAGAATCCACACAGGCCGCGACCCCATATCGGGACGCTCGTCCAT
      TACGCTTCTTAGGTGTCCGGCGCTGGGGTATAGCCCCTGCGAGCAGGTA

        V  D  A  G  R  S  A  I  G  T  F  G  G  S  L  S
3201  CGTCGACGCCGGCCGCAGTGCCATCGGAACCTTCGGCGGCAGTCTGTCG

        R  T  G  L  A  P  E  Q  I  D  E  V  I  L  G  Q  V
3301  CGTACCGGACTCGCGGCCGGAACAGATCGACGAGGTGATTCTCGGCCAGG

        G  L  P  H  S  V  P  A  M  T  I  N  K  V  C  G
3401  CGGGGCTACCGCATTCGGTGCCGGCCATGACCATCAACAAGGTCTGCGG
```

FIG.5(10/20)

```
CTAGGCCCCGCCGCCCGCGGTACCGAAAGGTGGTGACTAGCTACCGGCGGA 5'
L  D  P  A  A  P  A  M  A  K  W  W  Q  D  I  A  A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT 5'
 R  S  M  D  T  W  N  D  W  Y  K  R  Q  L  E  L  W

                                      "-35"
CGCGTGGTGTTGGGCGAACGGTATCATGTGGATTTGTGCACTGCAACAAAG 3'
GCGCACCACAACCCGCTTGCCATAGTACACCTAAACACGTGACGTTGTTTC 5'
          <----mRNA     "-10"                  "-35"

                                    phbA ------>
                   S/D              M  S  E  N  I  V  I
TTCCAATCAGAATCCAGAGGACATCCACGCCATGAGCGAGAACATCGTCAT 3'
AAGGTTAGTCTTAGGTCTCCTGTAGGTGCGGTACTCGCTCTTGTAGCAGTA 5'


 S  L  S  A  T  E  I  G  T  A  V  L  K  G  L  L  A
TCACTCTCGGCCACCGAGATCGGCACCGCCGTGCTCAAGGGGCTGCTGGCG 3'

  L  T  A  G  V  G  Q  N  P  A  R  Q  T  T  L  H  A
TGCTGACCGCCGGCGTGGGCCAGAACCCCGCCCGTCAGACCACGCTGCACG 3'

 S  G  L  K  A  V  H  L  A  M  Q  A  I  A  C  G  D
CAGCGGTCTGAAGGCGGTGCATCTGGCGATGCAGGCCATCGCCTGCGGGGA
```

FIG.5 (11/20)

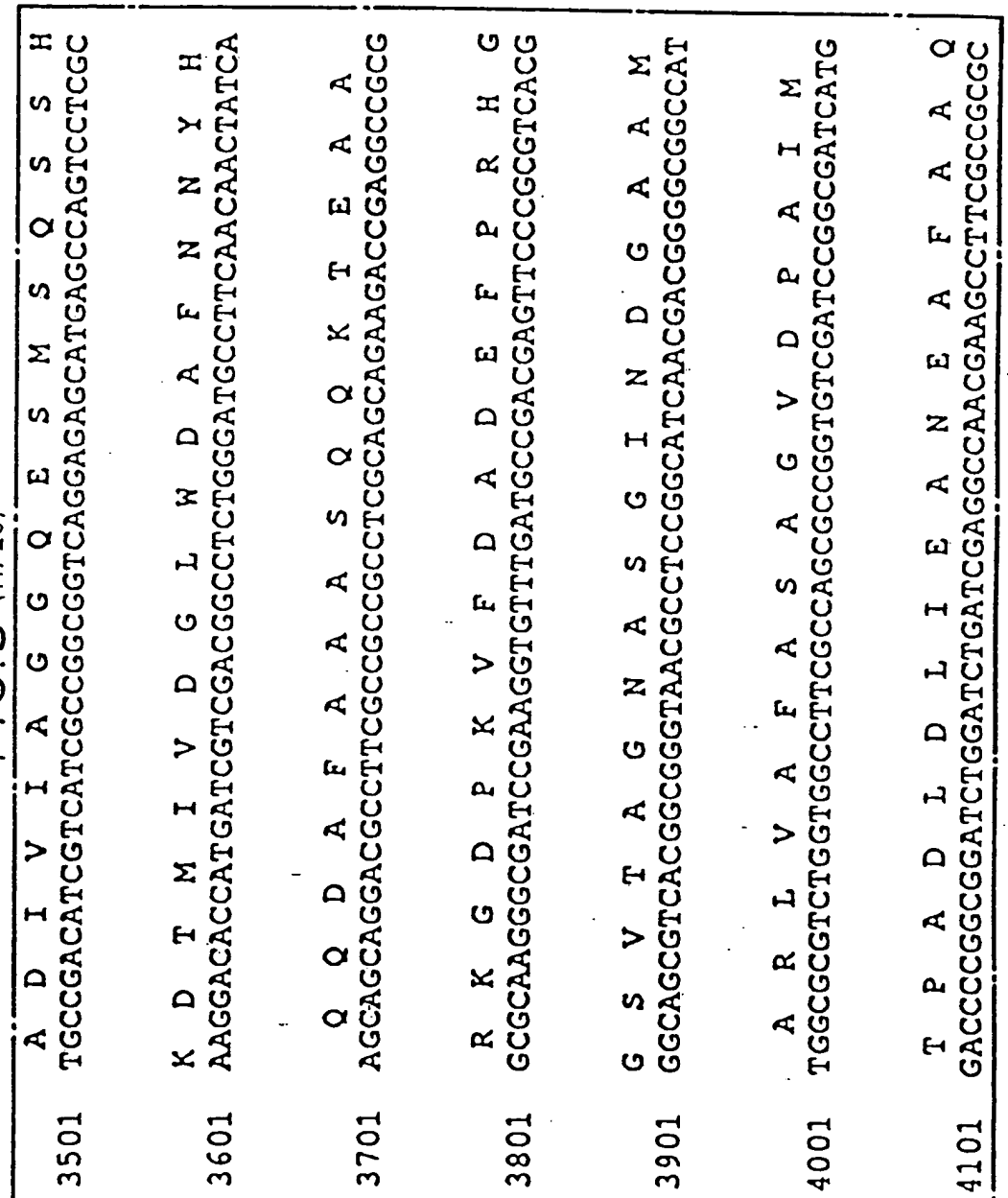

A D I V I A G G Q E S M S Q S S H
3501 TGCCGACATCGTCATCGCCGGCGGTCAGGAGAGCATGAGCCAGTCCTCGC
K D T M I V D G L W D A F N N Y H
3601 AAGGACACCATGATCGTCGACGGCCTCTGGGATGCCTTCAACAACTATCA
Q Q D A F A A A S Q Q K T E A A
3701 AGCAGCAGGACGCCTTCGCCGCCGCCTCGCAGCAGAAGACCGAGGCCGCG
R K G D P K V F D A D E F P R H G
3801 GCGCAAGGGCGATCCGAAGGTGTTTGATGCCGACGAGTTCCCGCGTCACG
G S V T A G N A S G I N D G A A M
3901 GGCAGCGTCACGGCGGGTAACGCCTCCGGCATCAACGACGGGGCGGCCAT
A R L V A F A S A G V D P A I M
4001 TGGCGCGTCTGGTGGCCTTCGCCAGCGCCGGTGTCGATCCGGCGATCATG
T P A D L L I E A N E A F A A Q
4101 GACCCCGGCGGATCTGGATCTGATCGAGGCCAACGAAGCCTTCGCCGCGC

FIG.5 (12/20)

```
V L P R S R D G Q R M G D W S M
ACGTCCTGCCGCGTTCGCCGACGGTCAGCGCATGGGCGACTGGTCGATG

M G T T A E N I A Q K Y G F T R E
CATGGGCACCACCGCCGAGAACATCGCCCAGAAGTACGGCTTACGCGCG

Q K A G R F Q D E I I P I E I P Q
CAGAAGGCTGGCCGCTTCCAGGACGAGATCATTCCGATCGAGATTCCGCA

T T A E S L G K L R P A F S K D
GCACCACGGCCGAGAGTCTGGGCAAGCTGCGTCCGGCCTTCTCGAAGGAC

V V V M K E S K A K E L G L K P M
GGTCGTGGTGATGAAGGAGTCCAAGGCCAAGGAACTGGGTCTGAAGCCGA

G T G P I P A S T K C L E K A G W
GGGACGGGCCCGATCCCGGCGTCGACCAAGTGCCTGGAGAAGGCCGGCTG

A M S V N Q D M G W D L S K V N
AGGCCATGTCGGTCAACCAGGACATGGGCTGGGATCTGTCCAAGGTCAAC
```

FIG. 5 (13/20)

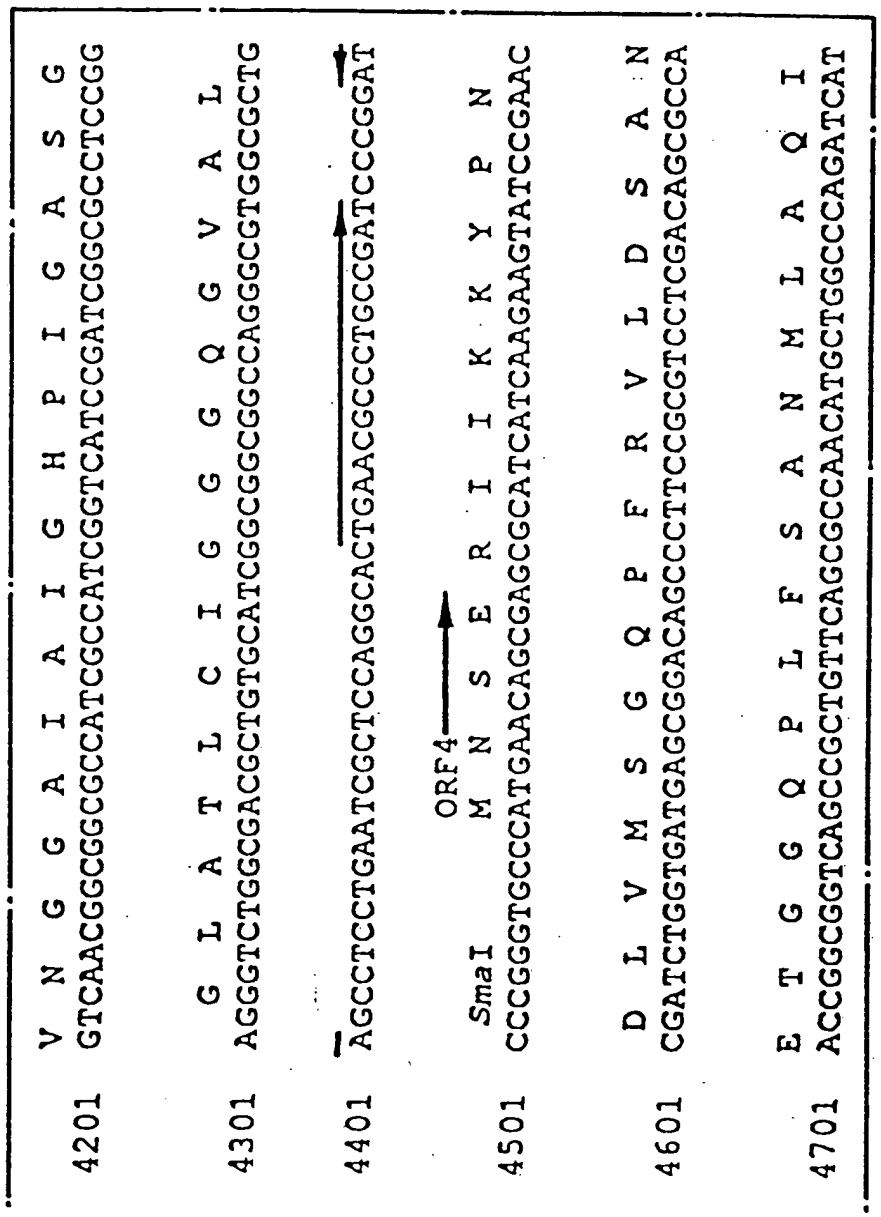

V N G G A I A I G H P I G A S G
4201 GTCAACGGCGGCGCCATCGCCATCGGTCATCCGATCGGCGCCTCCGG
G L A T L C I G G G Q G V A L
4301 AGGGTCTGGCGACGCTGTGCATCGGCGGCGGCCAGGGCGTGGCGCTG
4401 AGCCTCCTGAATCGCTCCAGGCACTGAACGGCCCTGCCGATCCCGGAT
ORF4
SmaI M N S E R I I K K Y P N
4501 CCCGGGTGCCCATGAACAGCGAGCGCATCATCAAGAAGTATCCGAAC
D L V M S G Q P F R V L D S A N
4601 CGATCTGGTGATGAGCGGACAGCCCTTCCGCGTCCTCGACAGCGCCA
E T G G Q P L F S A N M L A Q I
4701 ACCGGCGGTCAGCCGCTGTTCAGGCGCCAACATGCTGGCCCAGATCAT

FIG. 5 (14/20)

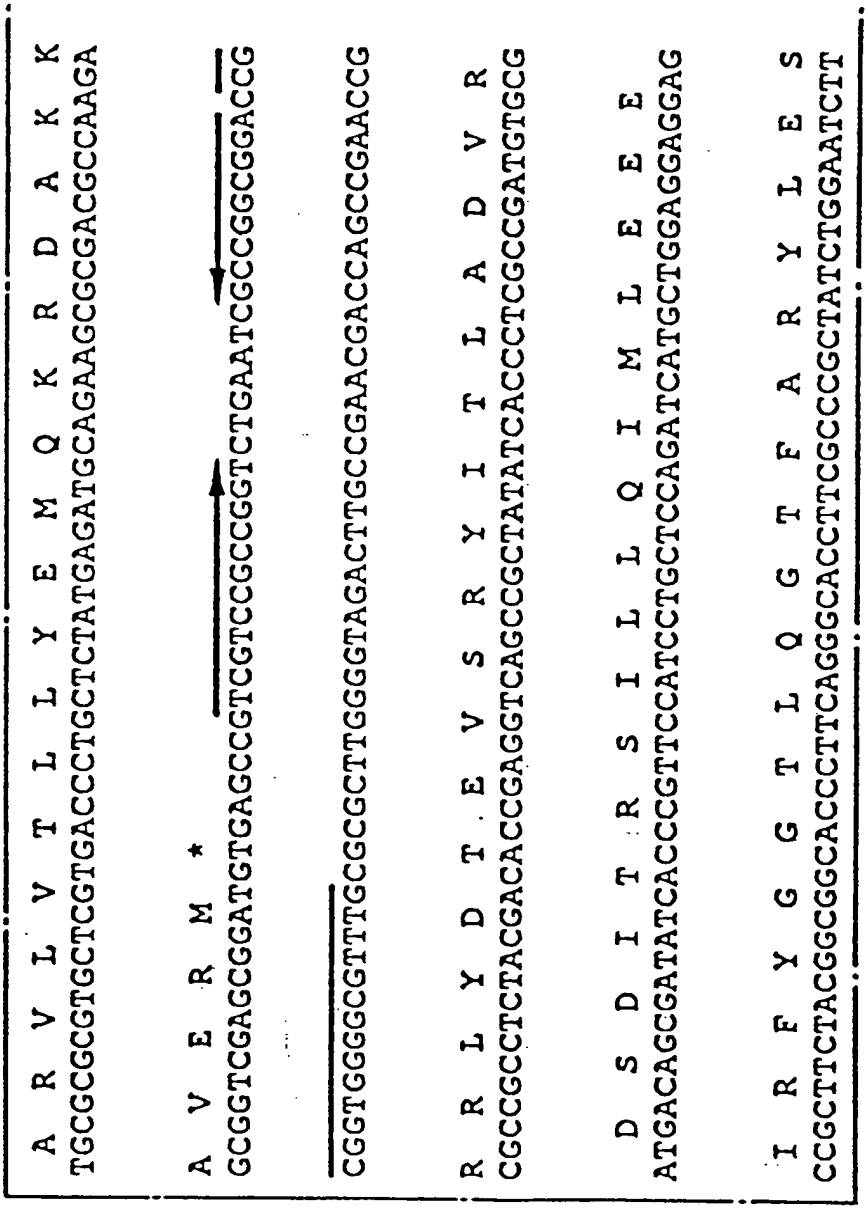

A R V L V T L L Y E M Q K K R D A K K
TGCGCGCGTGCTCGTGACCCTGCTCTATGAGATGCAGAAGCGCGACGCCAAGA
A V E R M *
GCGGTCGAGCGGATGTGAGCCGTCGTCCGCCGGTCTGAATCGCCGGCGGACCG
CGGTGGGGCGTTTGCGCGCTTGGGGTAGACTTGCCGAACGACCAGCCGAACCG
R R L Y D T E V S R Y I T L A D V R
CGCCGCCTCTACGACACCGAGGTCAGCCGCTATATCACCCTCGCCGATGTGCG
D S D I T R S I L L Q I M L E E E
ATGACAGCGATATCACCCGTTCCATCCTGCTCCAGATCATGCTGGAGGAGGAG
I R F Y G G T L Q G T F A R Y L E S
CCGCTTCTACGGCGGCACCCTTCAGGGCACCTTCGCCCGCTATCTGGAATCTT

FIG.5 (15/20)

```
                S  L  D  L  F  A  K  Q  Q  Q  E  V  T  K  A
4801  CACTCGACCTGTTCGCCAAGCAGCAACAGGAAGTGACCAAGGCACTC

                I  W  A  D  L  Q  D  E  L  M  R  A  A  G  F  P
4901  CTGGGCTGATCTCCAGGACGAACTCATGCGCGCGGCTGGCTTCCGG

                                "-10" (?)
5001  GCCGTCGGTCACAGCTTTATTGTGCAATGCAACATTGCTGCACTGCA

                E  W  T  N  K  S  V  E  R  M  T  S  F  G  E
5101  ACGAGTGGACCAACAAGAGCGTCGAGCGCATGACCAGCTTCGGTGAG

                L  Y  M  D  H  S  M  R  L  M  K  L  A  T  E  S
5201  CCTGTACATGGATCACAGCATGCGCCTGATGAAGCTGGCCACCGAGT

                S  E  R  V  M  A  E  S  K  A  T  M  Q  F  F  G
5301  AGCGAGCGCGTCATGGCCGAGAGGCAAGGCCACCATGCAGTTCTTCGG

                E  D  L  R  K  S  V  A  V  *
5401  GCGAAGATCTGCGCAAGAGCGTCGCCGTCTAAAGACGCCGACCTCTG
```

FIG.5 (16/20)

```
L T D N P F G T V T R L T Q K N V E
ACCGACAATCCCTTCGGGACGGTGACACGCCTGACTCAGAAGAACGTCGAGAT

V A P R K K E *                    "-35" (?)
TCGCGCCGCGCAAGAAAAAGAATAATGAGGATTGCGAAAATTGCGCTTGACG

                    ORF5 ⟵
                S/D  M N T T D S L K T V N
CAAACCTTACGGAGGAGATGATCATGAACACCACCGACAGCCTCAAGACCGTCA

L N V R L F E K L A A R Q M D A V N
CTGAACGTGCGTCTGTTCGAGAAGCTGGCCGCCCGTCAGATGGACGCCGTGAA

K G Y N D L F K G Q V D A T K E L
CCAAGGGTTACAATGACCTCTTCAAGGGTCAGGTCGACGCCACCAAGGAACTG

D A R D E Y R V W F E K S L N D V S
CGATGCCCGCGACGAATACCGCGTGTGGTTCGAGAAGAGCCTGAACGACGTCA

                ⟵                  ⟵
GGCCATCGCGATCCAGGGATGGATCGCCATTGGTCATGCTTCCGGATCGGCCG
```

FIG. 5 (17/20)

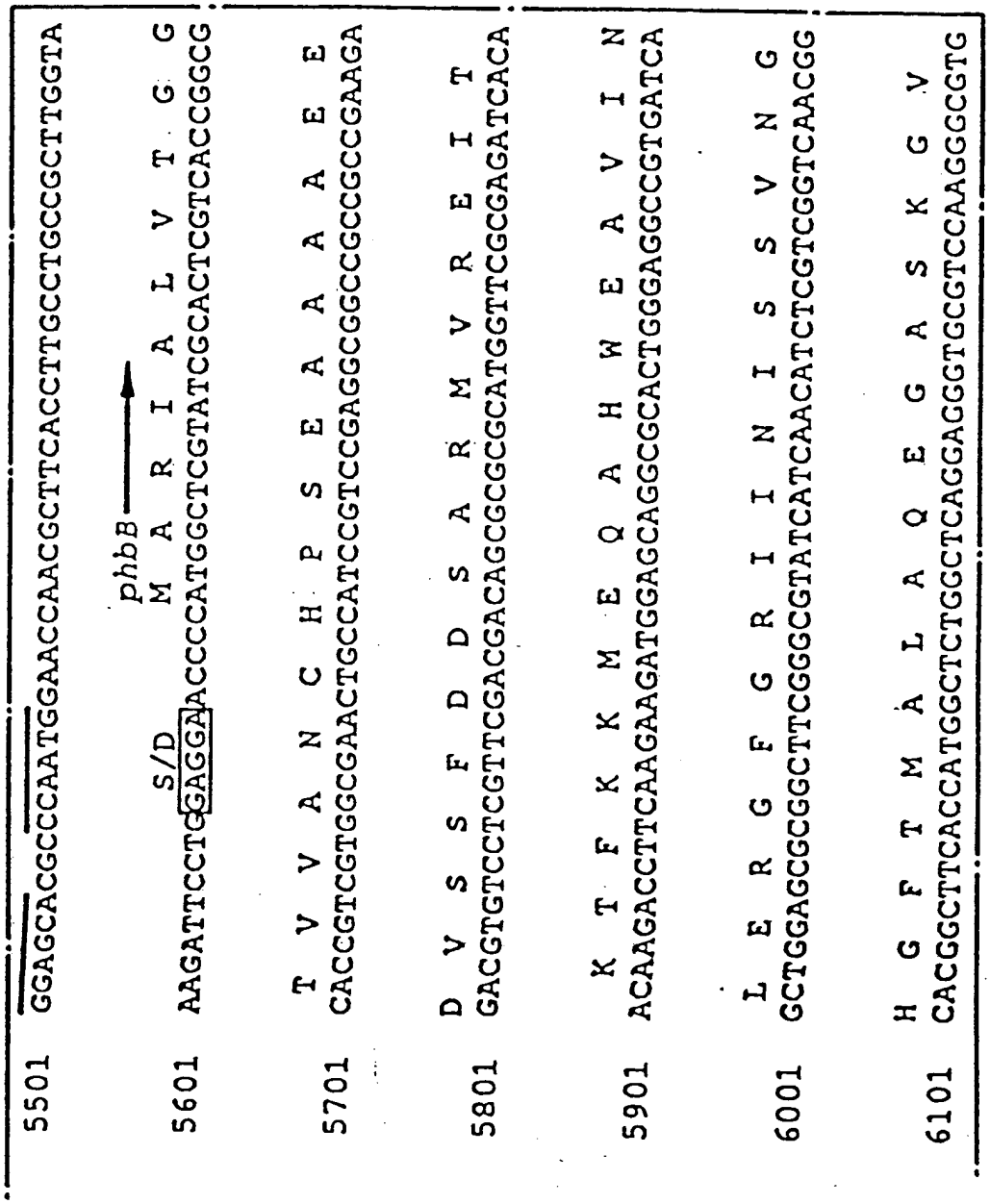

5501 GGAGCACGCCCAATGGAACCAACGCTTCACCTTGCCTGCCGCTTGGTA
S/D
phbB
5601 AAGATTCCTGGAGGAACCCCATGGCTCGTATCGCACTCGTCACCGGCG
M A R I A L V T G G
5701 CACCGTCGTGGCGAACTGCCATCCGTCCGAGGCGGCCGCCGCCGAAGA
T V V A N C H P S E A A A A E E
5801 GACGTGTCCTCGTTCGACGACAGCGCGCATGGTTCGCGAGATCACA
D V S S F D D S A R M V R E I T
5901 ACAAGACCTTCAAGAAGATGGAGCAGGCGCACTGGGAGGCCGTGATCA
K T F K K M E Q A H W E A V I N
6001 GCTGGAGGCGGCGGCTTCGGGCGTATCATCAACATCTCGTCGGTCAACGG
L E R G F G R I I N I S S V N G
6101 CACGGCTTCACCATGGCTCTGGCTCAGGAGGGTGCGTCCAAGGGCGTG
H G F T M A L A Q E G A S K G V

FIG. 5 (18/20)

```
GTAAAGTGGCCTTGAAGTTCGACGACACTGTTCATCGTTCTCAATAGTTCCA

  I  G  G  I  G  T  S  I  C  T  R  L  A  K  D  G  C
GCATCGGCGGCATCGGCACTTCGATCTGCACACGCCTGGCAAAGGATGGCTG

  W  K  Q  A  R  A  A  E  G  F  D  I  A  V  F  T  A
GTGGAAGCAGGCCCGTGCCGCCGAGGGGTTCGACATCGCCGTCTTCACCGCT

  E  Q  V  G  P  I  D  I  L  V  N  C  A  G  I  T  R  D
GAGCAGGTCGGTCCCATCGACATCCTGGTCAACTGTGCCGGCATCACCCGCG

  V  N  L  N  S  V  F  N  V  T  R  Q  V  W  D  G  M
ACGTCAACCTCAACAGCGTCTTCAACGTCACCCGTCAGGTGTGGGACGGGAT

  Q  R  G  Q  F  G  Q  A  N  Y  S  A  A  K  A  G  M
TCAGCGCGGCCAGTTCGGTCAGGCCAACTATTCCGCCGCCAAGGCCGGTATG

  T  V  N  T  I  S  P  G  Y  V  E  T  A  M  T  L  A  M
ACCGTCAACACCATCTCGCCCGGCTATGTCGAGACGGCCATGACCCTGGCGA
```

FIG.5(19/20)

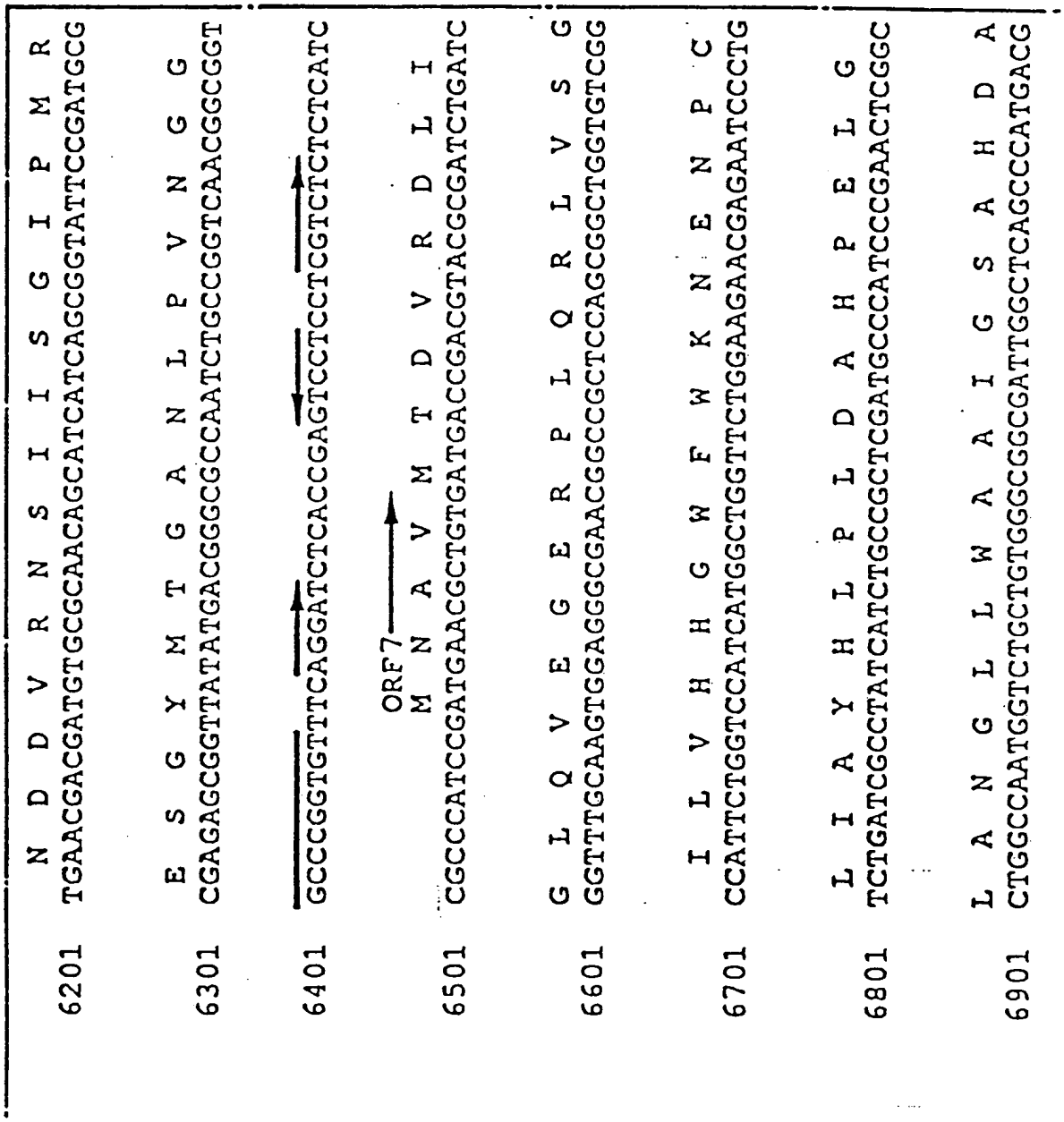

N D D V R N S I I S G I P M R
6201 TGAACGACGATGTGCGCAACAGCATCATCAGCGGTATTCCGATGCG
E S G Y M T G A N L P V N G G
6301 CGAGAGCGGTTATATGACGGGCGCCAATCTGCCGGTCAACGGCGGT
6401 GCCCGGTGTTTCAGGATCTCACCGAGTCCTCCTCGTCTCTCTCATC
ORF7
M N A V M T D V R D L I
6501 CGCCCATCCGATGAACGCTGTGATGACCGACGTACGCGATCTGATC
G L Q V E G E R P L Q R L V S G
6601 GGTTTGCAAGTGGAGGGCGAACGGCCGCTCCAGCGGCTGGTGTCGG
I L V H H G W F W K N E N P C
6701 CCATTCTGGTCCATCATGGCTGGTTCTGGAAGAACGAGAATCCCTG
L I A Y H L P L D A H P E L G
6801 TCTGATCGCCTATCATCTGCCGCTCGATGCCCATCCCGAACTCGGC
L A N G L L W A A I G S A H D A
6901 CTGGCCAATGGTCTGCTGTGGGCGGCGATTGGCTCAGCCCATGACG

FIG.5 (20/20)

```
R M A Q P N E I A A A I A F L A G D
TCGCATGGCTCAGCCTAATGAGATCGCCGCCGCCATCGCTTTCCTGGCCGGCGA

L F M H *
CTGTTCATGCATTGATTTAGATCATACCGGGCCGAATACAAAACACTGACAATG

ATGAGACGTTTACAGCCCGGCGCCAGCCGGGCTTTTTTTTGTGTAGAATCGAAT

R Y C D D V L D A A R F A D Y A P N
CGCTACTGCGATGACGTGCTCGACGCGGCGCGCTTCGCCGACTATGCGCCGAAT

V T A S A A L I E A A I A E H A D A
GCGTGACGGCCAGCGCCGCGTTGATCGAGGCCGCGGATCGCGGAGCACGCCGACG

L I G I K G Q R A R T L L S A G V S
CCTGATCGGCATCAAGGGGCAGCGCGCAAGGACATTGCTCAGCGCGGGTGTGAG

N N A T L G R R L D F I D M E P T A
AACAATGCCACACTCGGTCGCCGGCTCGATTTCATCGACATGGAACCGACCGCA

C V L H G A C L A
CCTGCGTCCTTCACGGAGCATGTCTCGCATC
```

FIG.6 (1/4)

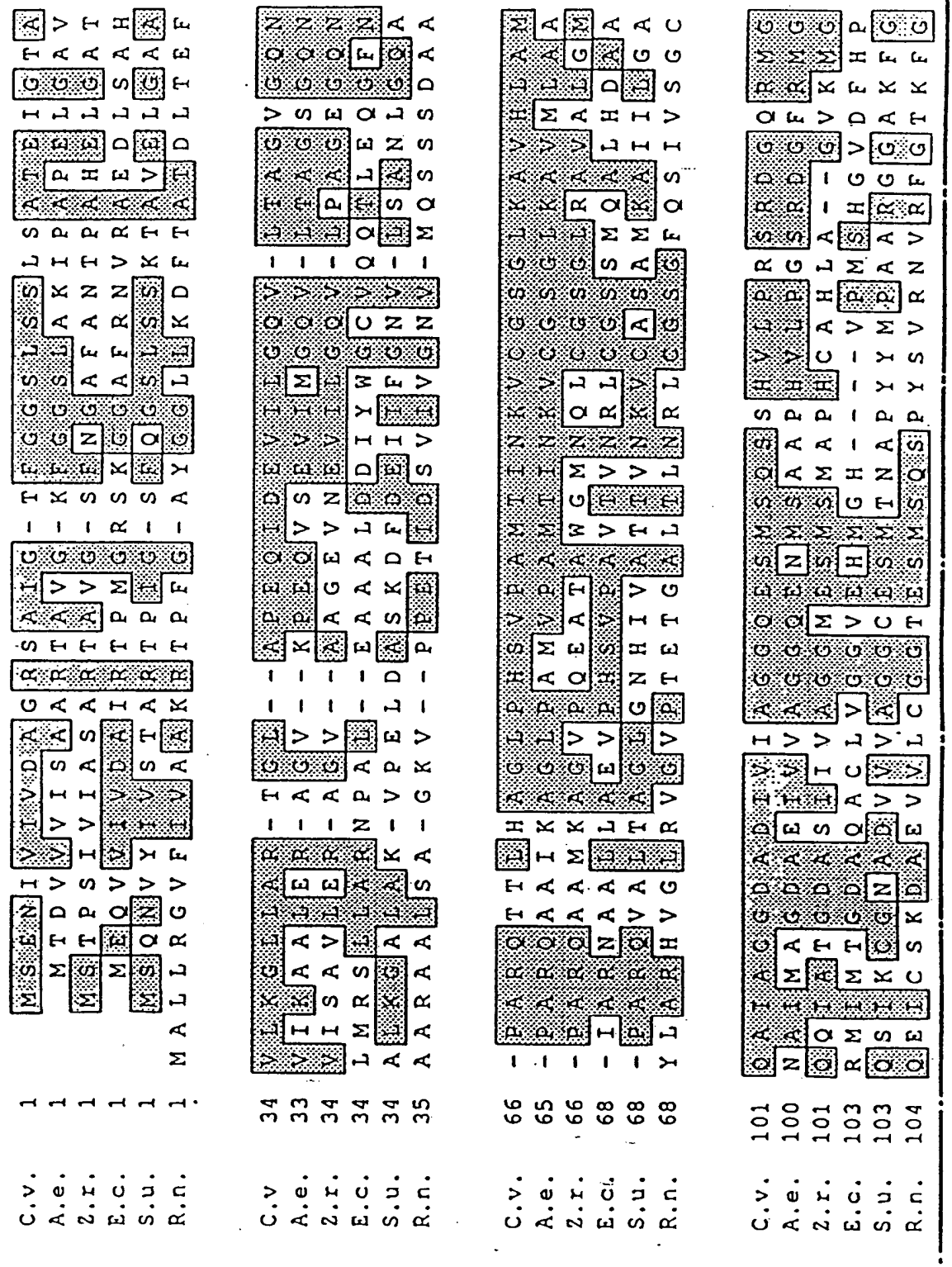


FIG.6 (2/4)

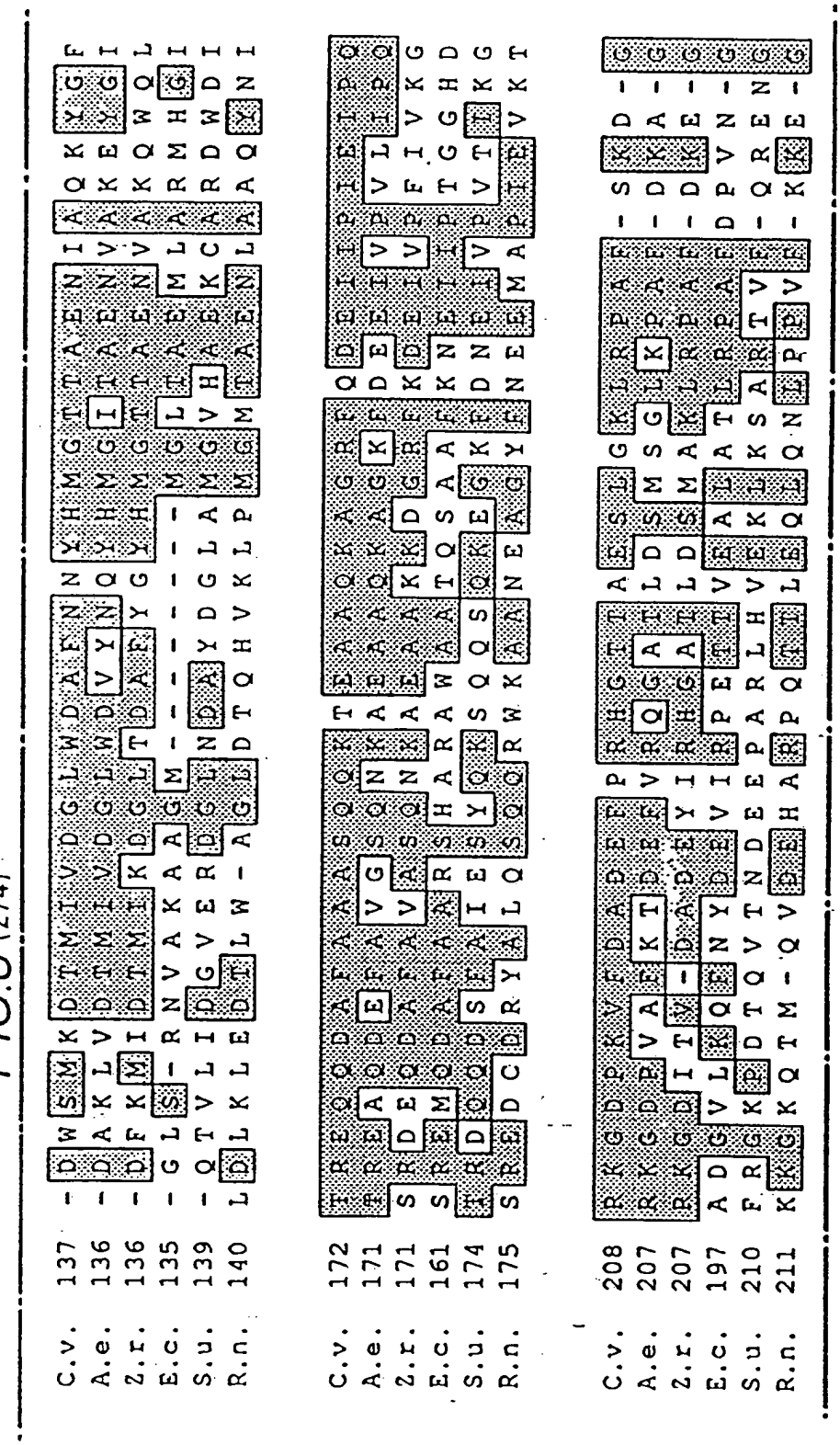

C.v. 137 - D W S M K D T M T V D G L W D A E N N Y H M G T T A E N I A Q K Y G F
A.e. 136 - D A K L V D T M T V D G L W D V Y N Q Y H M G I T A E N V A K E Y G I
Z.r. 136 - D F K M I D T M T K D G L T D A E Y G Y H M G T T A E N V A K Q W Q L
E.c. 135 - G L S - R N V A K A G M - - - - - - - - M G L T A E M L A R M H G I
S.u. 139 - Q T V L I D G V E R D G L N D A Y D G L A M G V H A E K C A R D W D I
R.n. 140 L D L K L E D T L W - A G E D T Q H V K L P M G M T A E N L A A Q Y N I
C.v. 172 T R E Q Q D A F A A A S Q Q K T E A A Q K A G R F Q D E I T P I E T P Q
A.e. 171 F R E A Q D E F A V G S Q N K A E A A Q K A G K F D E E T V P V L I P Q
Z.r. 171 S R D E Q D A F A V A S Q N K A E A A K K D G R F K D E F V P F I V K G
E.c. 161 S R E M Q D A F I A R S H A R A W A A T Q S A A F K N E I I P P T G G H D
S.u. 174 I R D Q Q D S E A I E S Y Q K S Q Q S Q R E G K F D N E I V P P V T I K G
R.n. 175 S R E D C D R Y A L Q S Q Q R W K A A N E A G Y F N E E M A P I E V K T
C.v. 208 R K G D P K V F D A D E F P R H G T T A E S L G K L R P A E - S K D - G
A.e. 207 R K G D E V A E K T D E F V R Q G A T L D S M S G L K P A E - D K A - G
Z.r. 207 R K G D I T V - D A D E Y I R H G A T L D S M A K L R P A E - D K E - G
E.c. 197 A D G V L K Q E N Y D E V I R P E T T V R A T L A T L R P A E D P V N - G
S.u. 210 F R G K P D T Q V T N D E E P A R L H V E K D K S A R T V E - Q R E N G
R.n. 211 K K G K Q T M - Q V B E H A R P Q T T L E Q N L P P V E - K K E - G

FIG.6 (3/4)

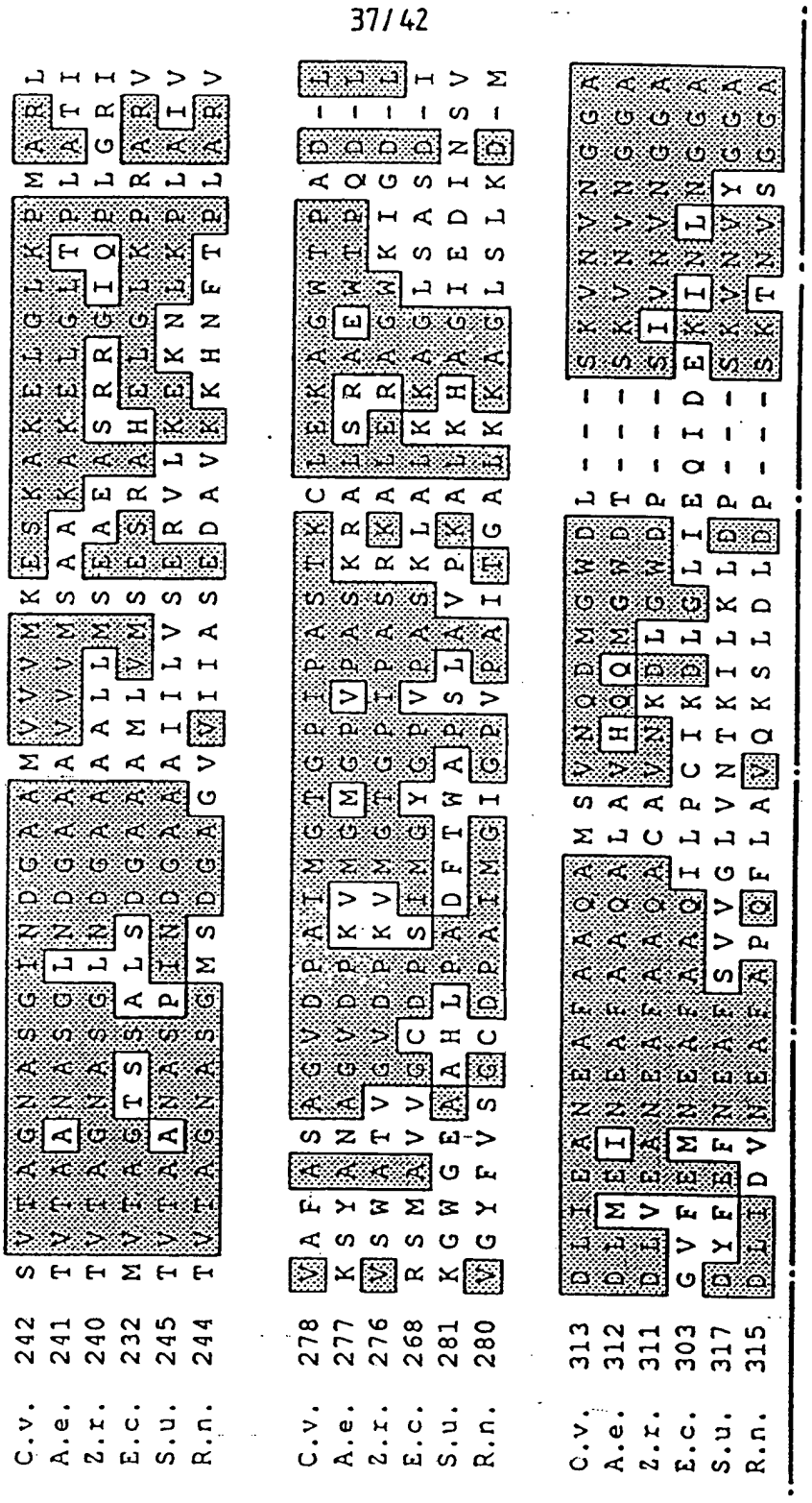
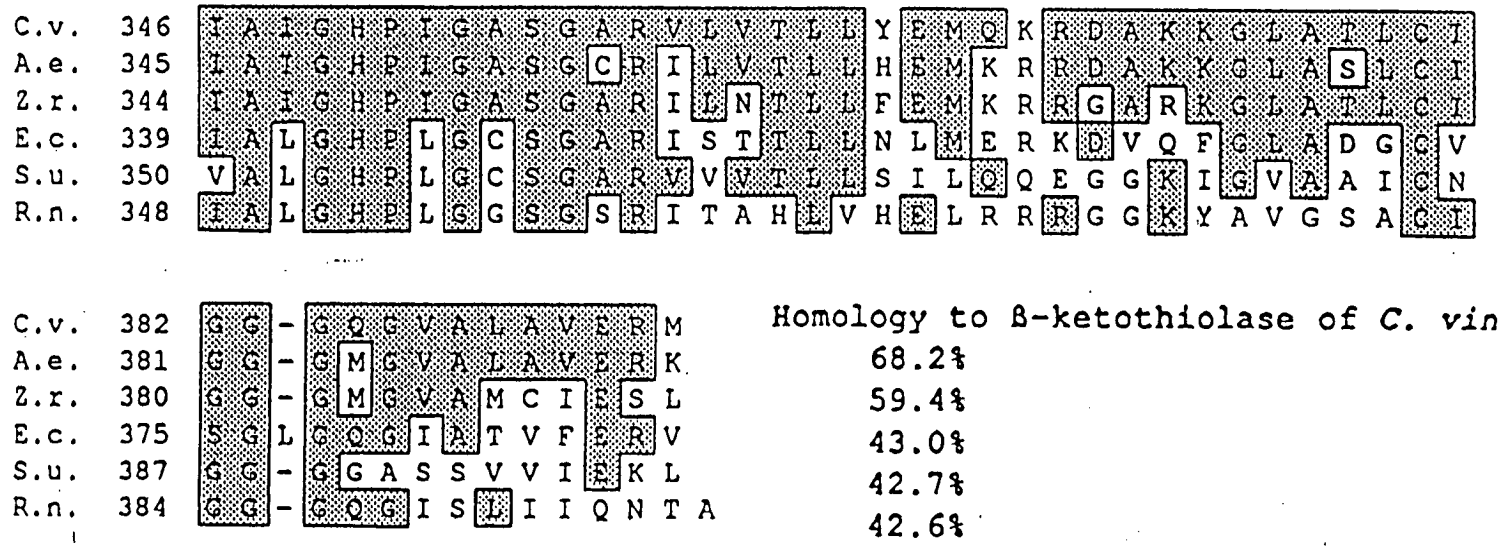

C.v. 242 S V T A G N A S G I N D G A A M V V V M K E S K A K E L G L K P M A R L
A.e. 241 T V T K A N A S G L N D G A A A V V V M S A A K A K E L G L T P L A T I
Z.r. 240 T V T T A G N A S G L N D G A A A A L L M S E A E A S R R G I Q P L G R I
E.c. 232 M V T T R G T S S A L S D G A A A M L V M S E S R A H E T L G L K P R A R V
S.u. 245 T V T T A A N A S P I N D G A A A I I L V S E R V L K E K N L K P L A I V
R.n. 244 T V T A G N A S G M S D G A G V V I I A S E D A V K K H N F T P L A R V
C.v. 278 V A F A S A G V D P A I M G T G P I P A S T K C L E K A G W T P A D - L
A.e. 277 K S Y A A N A G V D P K V N G M G P V P A S K R A L S R A E W T P Q D - L
Z.r. 276 V S W A T V G V D P K V M G T G P I P A S R K A L E R A G W K I G D - L
E.c. 268 R S M A S V V G C D P S I M G Y G P V P A S K L A L K A G L S A S D - I
S.u. 281 K G W G E A A H L P A D F T W A P S L A V P K A L K H A G I E D I N S V
R.n. 280 V G Y F V S G C D P A I M G I G P V P A I T G A L K K A G L S L K D - M
C.v. 313 D L I E A N E A F A A Q A M S V N Q D M G W D L - - - S K V N V N G G A
A.e. 312 D L M E I N E A F A A Q A L A V H Q Q M G W D T - - - S K V N V N G G A
Z.r. 311 D L V E A N E A F A A Q A C A V N K D L G W D P - - - S I V N V N G G A
E.c. 303 G V F E M N E A F A A Q I L P C I K D L G L I E Q I D E K I N L N G G A
S.u. 317 D Y F E F N E A F S V V G L V N T K I L K L D P - - - S K V N V Y G G A
R.n. 315 D L I D V N E A F A P Q F L A V Q K S L D L D P - - - S K T N V S G G A

FIG.6 (4/4)



Homology to ß-ketothiolase of *C. vinosum*:

	68.2%
	59.4%
	43.0%
	42.7%
	42.6%

Comparison of amino acid sequences of the ß-ketothiolases encoded by *Chromatium vinosum* (C.v.), *Alcaligenes eutrophus* (A.e.), *Zoogloea ramigera* (Z.r.), *Escherichia coli* (E.c.), *Saccharomyces uvarum* (S.u.) and *Rattus norvegicus* (R.n.).

FIG. 7 (1/2)

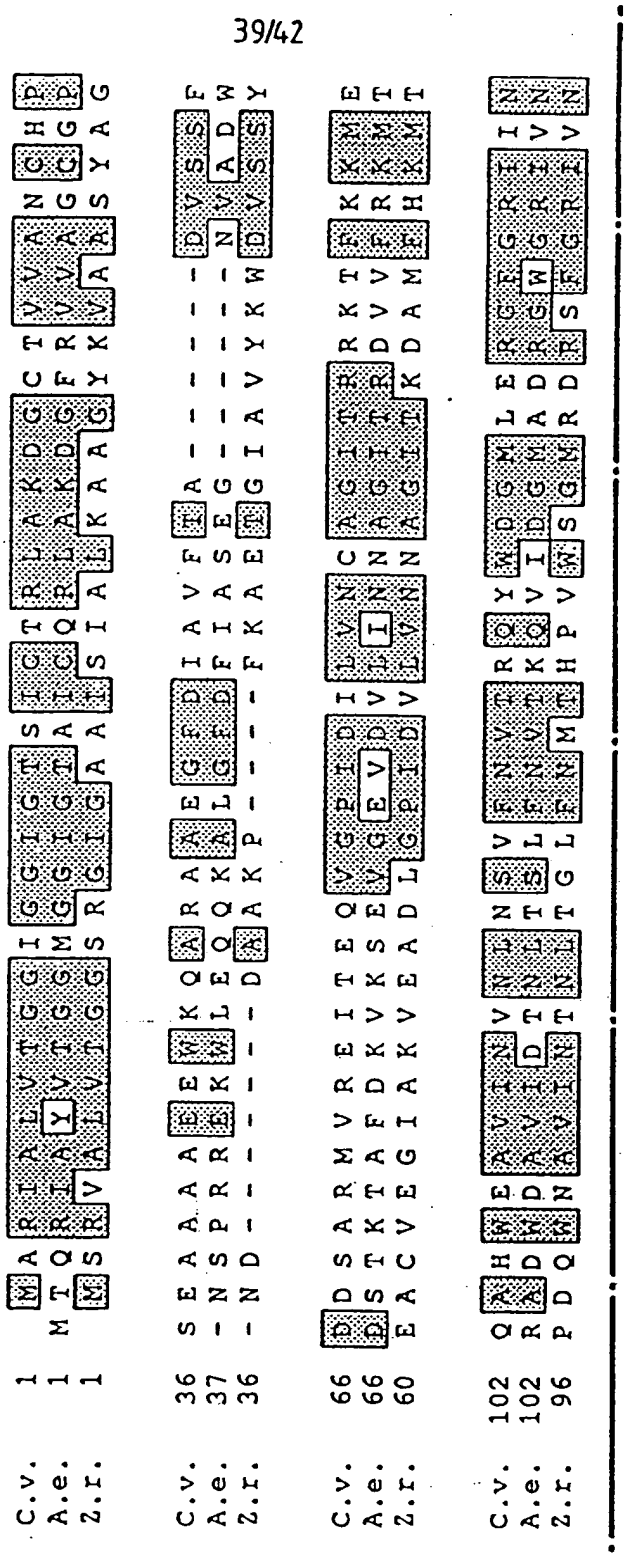
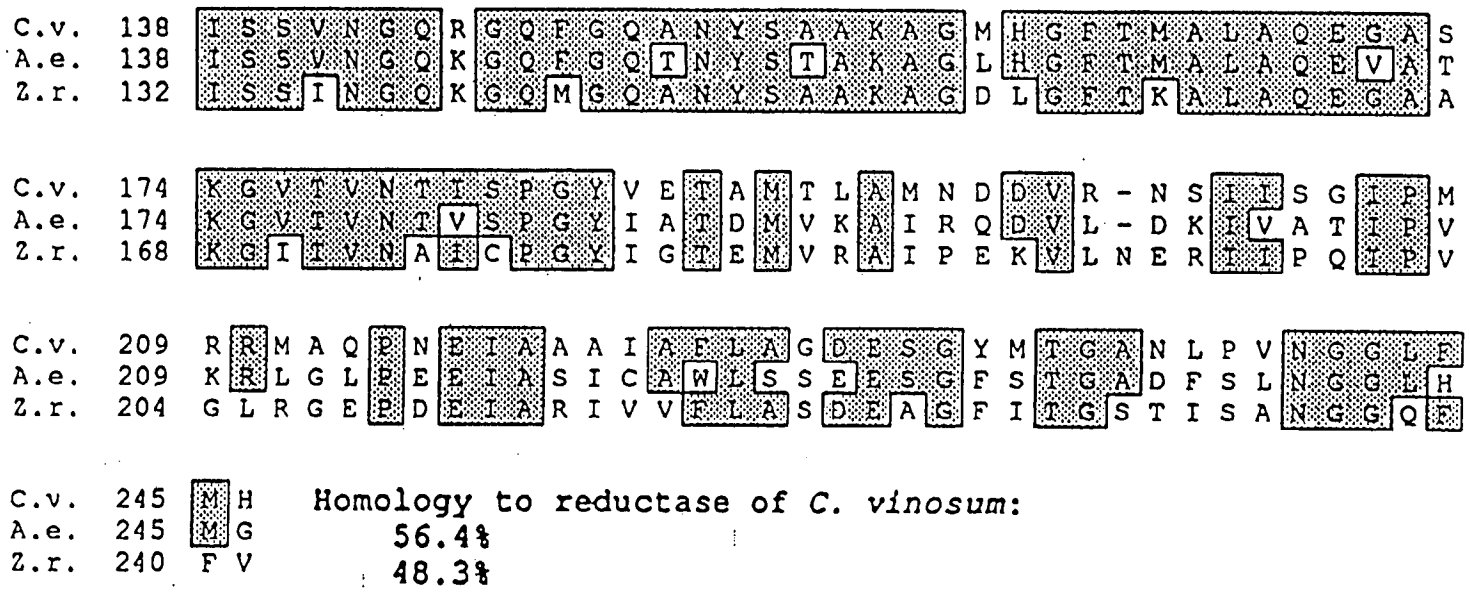


FIG. 7 (2/2)



Comparison of amino acid sequences of reductases encoded by *C. vinosum* (C.v.), *A. eutrophus* (A.e.) and *Z. ramigera* (Z.r.).

FIG.8 (1/2)

Strain (plasmid)	relevant markers	Medium
S17-1 (pHP1014)	none	LB-Tc-Glu
S17-1 (pHP1014::PP10)	*phbA*$^+$, *phbB*$^+$, *phbC*$^+$, ORF2$^+$	LB-Tc-Glu
S17-1 (pHP1014::EP94)	*phbA*$^+$, *phbB*$^+$, *phbC*$^+$, ORF2$^+$	LB-Tc-Glu
S17-1 (pSUP202)	none	LB-Tc-Glu
S17-1 (pSUP202::PP10)	*phbA*$^+$, *phbB*$^+$, *phbC*$^+$, ORF2$^+$	LB-Tc-Glu
XL1-Blue (KS$^-$)	none	LB-Ap-Glu
XL1-Blue (KS$^-$)	none	LB-Ap-IPTG
XL1-Blue (KS$^-$::SE45+)	*phbA*$^+$, *phbB*$^-$, *phbC*$^+$, ORF2$^+$	LB-Ap-Glu
XL1-Blue (KS$^-$::SE45+)	*phbA*$^+$, *phbB*$^-$, *phbC*$^+$, ORF2$^+$	LB-Ap-IPTG
XL1-Blue (KS$^-$::SE45-)	*phbA*$^+$, *phbB*$^-$, *phbC*$^+$, ORF2$^+$	LB-Ap-Glu
XL1-Blue (KS$^-$::SE45-)	*phbA*$^+$, *phbB*$^-$, *phbC*$^+$, ORF2$^+$	LB-Ap-IPTG
XL1-Blue (KS$^-$::B55)	*phbA*$^+$, *phbB*$^+$, *phbC*$^-$, ORF2$^+$	LB-Ap-Glu
XL1-Blue (KS$^-$::B55)	*phbA*$^+$, *phbB*$^+$, *phbC*$^-$, ORF2$^+$	LB-Ap-IPTG

FIG. 8 (2/2)

Specific activity (U/g of protein)				Accumulation of PHB (% of cellular dry weight)
		Acetoacetyl-CoA reductase		
PHB synthase	ß-Ketothiolase	NADH-dependent	NADPH-dependent	
<0.1	<20	<20	<20	<0.1
6.1	1190	420	60	10.2
5.2	940	310	60	9.7
<0.1	<20	<20	<20	<0.1
6.1	1320	320	60	12.1
<0.1	<20	<20	<20	<0.1
<0.1	<20	<20	<20	<0.1
6.0	1120	<20	<20	<0.1
4.3	980	<20	<20	<0.1
4.8	470	<20	<20	<0.1
2.9	70	<20	<20	<0.1
<0.1	80	30	<20	<0.1
<0.1	1870	610	40	<0.1

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01291

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/52; C12N15/53; C12N15/54; C12N1/21
A01H5/00; C12P7/62

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12P ; A01H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category*	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	ARCHIVES OF MICROBIOLOGY vol. 155, no. 5, 1991, pages 415 - 421 LIEBERGESELL, M., ET AL. 'Formation of ploy-3 hydroxyalkanoates by phototrophic and chemolithic bacteria' see the whole document	1-12
A	TRENDS IN BIOTECHNOLOGY vol. 5, no. 9, September 1987, pages 246 - 250 BYROM, D. 'Polymer synthesis by microorganisms: tecnology and economics' see page 248, left column; table 2	1-12
A	WO,A,9 100 917 (MIT) 24 January 1991 see the whole document	1-12

-/--

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 OCTOBER 1992	19. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MADDOX A.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,8 900 202 (MIT) 12 January 1989 see the whole document	1-12
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 26, 15 September 1989, BALTIMORE, MD US pages 15298 - 15303 PEOPLES, O.P., ET AL. 'Poly-beta-hydroxybutyrate (PHB) boisynthesis in Alcaligenes eutrophus H16' see figure 4	1-12
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 26, 15 September 1989, BALTIMORE, MD US pages 15293 - 15297 PEOPLES, O.P., ET AL. 'Poly-beta-hydroxybutyrate biosynthesis in Alcaligenes eutrophus H16' see figures 5,6	10,12
A	SCIENCE vol. 245, 15 September 1989, LANCASTER, PA US pages 1187 - 1189 POOL, R., ET AL. 'In search of the plastic potato' see page 1189, column 2 - column 3	8
A	J. BACTERIOLOGY vol. 170, no. 12, December 1988, pages 5837 - 5847 SCHUBERT, P., ET AL. 'Cloning of the alcaligenes eutrophus genes for th synthesis of PHB in Escherichia coli' see the whole document	1-12

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201291 SA 62148

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9100917	24-01-91	EP-A- 0482077	29-04-92
WO-A-8900202	12-01-89	EP-A- 0329770	30-08-89